GATA 3 EXPRESSION IN BREAST CARCINOMA, AND ITS ASSOCIATION WITH ER, PR, HER 2NEU STATUS, A 3 YEARS STUDY IN A TERTIARY CARE CENTRE

Dissertation submitted in

partial fulfillment of the requirements for the degree of

M.D. PATHOLOGY

BRANCH- III

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

CHENNAI

MAY 2019

CERTIFICATE

This is to certify that this dissertation entitled**,** "**GATA 3 EXPRESSION IN BREAST CARCINOMA, AND ITS ASSOCIATION WITH ER, PR, HER 2NEU STATUS, A 3 YEARS STUDY IN A TERTIARY CARE CENTRE"** in partial fulfillment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R. Medical University to be held in May 2019.

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DECLARATION

I, **Dr. Rituporna Boruah,** solemnly declare that the dissertation entitled **"GATA 3 EXPRESSION IN BREAST CARCINOMA, AND ITS ASSOCIATION WITH ER, PR, HER 2 NEU STATUS, A 3 YEARS STUDY IN A TERTIARY CARE CARE CENTRE''** is the bonafide work done by me at the Institute of pathology, Madras Medical College under the expert guidance and supervision of **Prof. Dr. Rajavelu Indira, M.D., Professor of Pathology, Govt. Kasturba Gandhi Hospital and Dr. Vijaya Baskar, MD Assistant professor of Pathology, Institute Of Pathology, Madras Medical College, Chennai.** The dissertation is submitted to the Tamilnadu Dr. M.G.R Medical University towards partial fulfilment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Dr. RITU PORNA BORUAH

Place : Chennai

Date :

ACKNOWLEDGEMENT

I express my sincere thanks to Prof.Dr.R.Jayanthi, MD, FRCP, Dean, Madras Medical College and Rajiv Gandhi Government General Hospital, for permitting me to utilize the facilities of the institution.

I take this opportunity to express my thanks and heartfelt gratitude to **Prof. Dr.Bharathi Vidhya Jayanthi, M.D.,** Director and Professor of Pathology, Institute of pathology, Madras Medical College for her keen interest, constant encouragement and valuable suggestions throughout the study.

I am extremely thankful to my guide **Prof. Dr. Rajavelu Indira, M.D., Professor of Pathology, Govt. Kasturba Gandhi Hospital** for valuable suggestions , guidance and encouragements throughout the study.

I am also extremely thankful to **Prof.Dr.R.Padmavathi M.D., Prof.Dr.Ramamoorthy M.D., Prof.Dr.Geetha Devadas M.D., Prof.Dr.Sudha Venkatesh M.D., Prof.Dr.M.P.Kanchana M.D., Prof.Dr.K.Rama M.D., Prof.Dr.S.Pappathi M.D., Prof.Dr.Selvambigai M.D.,** for their valuable suggestions and encouragement throughout the study.

I would also like to thank **Dr. Vijaya Baskar, MD Assistant Professor** who helped me a lot in finalizing thesis work within the limited time frame.

I express my heartfelt thanks to all my assistant professors for their help and suggestions during the study.

I also would like to thank the Institutional Ethics Committee for approving my study.

I am thankful to the statistician for the help in statistical analysis.

I thank my friends, colleagues, senior & junior postgraduates, technicians and staff for their continuous support and helpful advice.

On a personal note, I extend my gratitude to all the members of my family for their constant support.

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Dear Dr.Rituporna Boruah,

The Institutional Ethics Committee has considered your request and approved your study titled **"GATA 3 EXPRESSION IN BREAST CARCINOMA, AND ITS ASSOCIATION WITH ER, PR, HER 2NEU STATUS – A 3 YEARS STUDY IN A TERTIARY CARE CENTRE " - NO.25122017**

The following members of Ethics Committee were present in the meeting hold on **05.12.2017** conducted at Madras Medical College, Chennai 3

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We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

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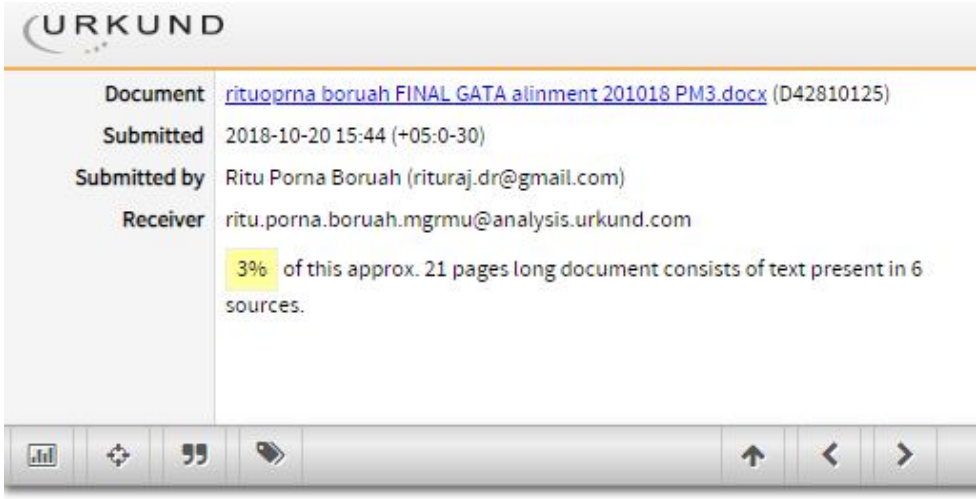

INTRODUCTION Malignancy of breast is a worldwide major health problem. . Every year more than a million women are newly diagnosed as carcinoma of breast. As advancing the medical science the mortality from the carcinoma breast is beginning to fall because of early diagnosis and improved treatment facilities.[1] From various studies it is established the breast cancer is rare before the age of 25 years, but incidence increases with age from the third decade of life.[94] Carcinoma of breast is seen most commonly in females of reproductive age groups, but risk of developing breast carcinoma is increased with age. In males it is very rare . The incidence of breast carcinoma is in male is less than 1%. The pathogenesis of breast carcinoma is multifactorial, depend on a combination of genetic factor, race ,family history, reproductive history etc. Most of the malignant tumors of breast are adenocarcinomas (more than 95% cases). They first arise from duct or lobular system from the precursor lesions (carcinoma in situ).[94] Malignant stromal tumor can also occur in breast. Angiosarcoma is the commonest stromal malignancy in breast. Other stromal malignant lesions of breast are malignant phyllodes tumor, Rhabdomyosarcoma, liposarcoma, leiomyosarcoma, chondrosarcoma and osteosarcoma.[94] Morphologically malignant lesions of breast tissue are divided on the basis of 2 characteristics' (1) Depending on invasion to the stroma In situ carcinoma tumors are confined to the epithelial component. Invasion of the stroma by the tumor component is termed as invasive carcinoma. (2)

PLAGIARISM CERIFICATE

This is to certify that this dissertation work titled **"GATA 3 EXPRESSION IN BREAST CARCINOMA, AND ITS ASSOCIATION WITH ER, PR, HER 2NEU STATUS, A 3 YEARS STUDY IN A TERTIARY CARE CENTRE"** of the candidate **Dr. RITU PORNA BORUAH** with registration number **201613008** for the award of **M.D PATHOLOGY** (Branch-III). I personally verified the urkund.com website for the purpose of Plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion and result shows 3% of plagiarism in the dissertation.

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ABBREVIATIONS

H&E	-	Hematoxylin and Eosin
IHC	-	Immunohistochemistry
ER	-	Estrogen Receptor
PR	-	Progesterone Receptor
HER 2	-	Human Epidermal Growth Factor Receptor- 2
HRP	-	Horse radish peroxidase
WHO	-	World Health Organization
FISH	-	Fluorescence in situ hybridization
DAB	-	Diamino benzidine
IDC	-	Invasive Ductal Carcinoma
IBC	-	Invasive Breast Carcinoma
ILC	-	Invasive lobular Carcinoma
NST	-	No Special type
MRM	-	Modified Radical Mastectomy
TDLU	-	Terminal Duct–Lobular Unit
IDC NST	-	Invasive ductal carcinoma no special type
Ca	-	Carcinoma
LN	-	Lymph Node
GATA	-	Family of transcription factor characterized by ability to bind with the " GATA" sequence of DNA.

CONTENTS

INTRODUCTION

Malignancy of breast is a worldwide major health problem. . Every year more than a million women are newly diagnosed as carcinoma of breast. Due to advances in medical science the mortality from the carcinoma breast is beginning to fall because of early diagnosis and improved treatment facilities.[1] From various studies it is established that breast cancer is rare before the age of 25 years, but incidence increases with age from the third decade of life.[94]

Carcinoma of breast is seen most commonly in females of reproductive age groups, but risk of developing breast carcinoma is increased with age. In males it is very rare . The incidence of breast carcinoma in male is less than 1%. The pathogenesis of breast carcinoma is multifactorial, depending on a combination of genetic factor, race ,family history, reproductive history etc.

Most of the malignant tumors of breast are adenocarcinomas (more than 95% cases). They first arise from duct or lobular system from the precursor lesions (carcinoma in situ).[94] Malignant stromal tumor can also occur in breast. Angiosarcoma is the commonest stromal malignancy in breast. Other stromal malignant lesions of breast are malignant phyllodes tumor, Rhabdomyosarcoma, liposarcoma, leiomyosarcoma, chondrosarcoma and osteosarcoma.[94]

Morphologically malignant lesions of breast tissue are divided on the basis of 2 characteristics'

(1) Depending on invasion in to the stroma

In situ carcinoma tumors are confined to the epithelial component. Invasion of the stroma by the tumor component is termed as invasive carcinoma.

(2) Whether it is ductal or lobular type[18]

For diagnosis of malignant tumor of breast, the lesions should be examined clinically, radiologically and by tissue sampling. The combination of these three is termed as triple assessment. Mammography and ultrasonography are the most commonly used radiological test for screening of breast carcinoma. Now a days many breast carcinoma cases are detected early by using mammogram. Most common symptoms of carcinoma breast is breast lump, with or without pain. Other symptoms are nipple discharge, nipple retraction or eczema mayalso occur.[95]

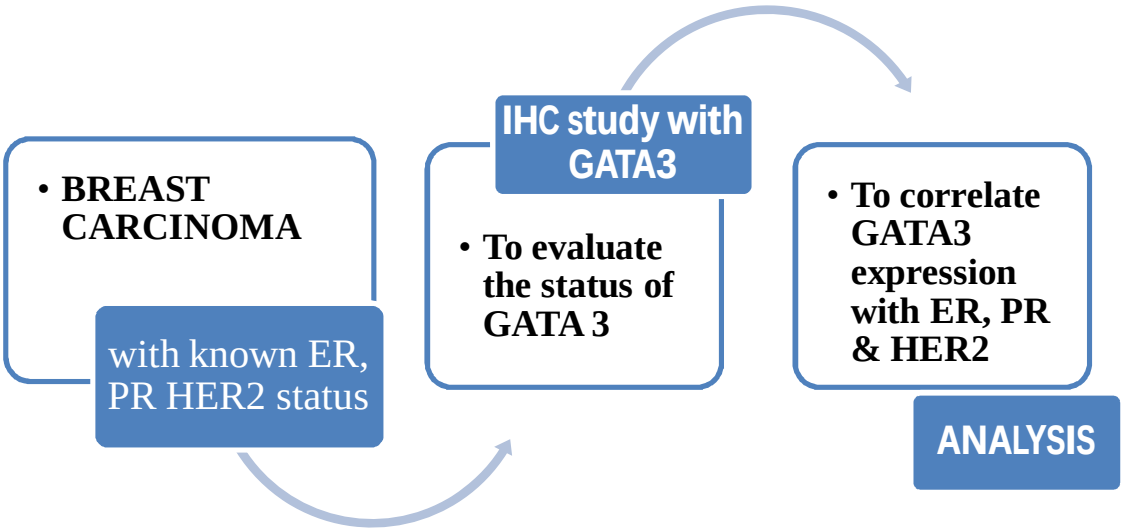
Like other malignant tumors, the prognosis of breast carcinoma also depends upon various factors, like age, tumor size, histologic grade, invasiveness, metastasis, molecular subtype etc. Breast carcinomas expressing estrogen receptor (ER) and progesterone receptor (PR) have excellent response to hormonal therapy. Expression of HER-2 is associated with poor prognostic outcome.

Some recent studies show that expression of GATA-3 gene is associated with good prognosis and survival in breast carcinoma patients. Few other studies has shown that expression of GATA-3 is limited to carcinoma of breast and carcinoma of urinary bladder and therefore this immunohistochemical marker may be a sensitive and specific marker for metastatic breast carcinomas particularly for those tumors which have lack of specific markers to prove mammary origin.[96]

AIMS & OBJECTIVES

To evaluate the expression of GATA-3 in breast carcinoma by using Mouse monoclonal antibody.

To compare the GATA-3 expression with already known ER, PR and HER 2 neu status of breast carcinoma cases.



REVIEW OF LITERATURE

EPIDEMIOLOGY

Breast carcinoma is the second most common malignancy after carcinoma lung worldwide. Among the women it is the most common cancer in the globe, contributing 25.4 % of total number of newly diagnosed cancer cases. [2]

It is rare in women below the age of 25 years but, after the age of 30 years the incidence increases rapidly. According to the Indian cancer, registry data carcinoma breast is the most common malignant tumor among the Indian ladies. The rate of incidence is 25.8 per 100,000 Indian women. Mortality from breast cancer is high in rural registries than urban registries of India. [3]

RISK FACTORS

For the development of breast carcinoma action of several risk factors have been well proved, and many others are yet to be established. [4]

1. Gender: Female gender is itself a risk factor of developing breast carcinoma. Approximately 99% of the breast carcinoma patients are female.

2. Age: Age is also a risk factor of breast carcinoma. Risk of developing breast carcinoma is increased with age .
3. Geographical Location: The incidence of Ca breast is high in western world (91.4 new cases per 100 000 women/year), but low in Asian and African countries.
4. Race/Ethnicity: Incidence of breast cancer are higher among the whites than other racial and ethnic groups.
5. Family History: Women who have a history of breast carcinoma in first-degree relatives are at 2 to 3 times higher risk than the general population. The most known susceptible genes for familial breast cancer are tumor suppressor genes BRCA1, BRCA2, TP53, and CHEK2. Approximately 3% of all breast cancers are due to mutations in *BRCA1* (chromosome 17q21) and *BRCA2* (chromosome13q12.3) .
6. Reproductive History: Breast Carcinoma is high among the women with early menarche, late menopause, nulliparity and late age at first child birth. Oophorectomized ladies before the age of 35 years have reduced incidence. Studies shows that the ladies who attended menarche before the age of 11 years have 20% increased risk of developing breast carcinoma than the ladies who attended menarche after the age of 14 years.

Pregnancy before the age of 20 years decreases the chances of developing breast carcinoma.

7. Lactation history also plays an important role in reducing the incidence of breast carcinoma.
8. Estrogens: Estrogen is also an important risk factor for breast carcinoma. Some recent studies shows that the women who use hormone replacement therapy for a longer duration are associated with an increased risk of carcinoma breast than in those who use estrogens alone.
9. Carcinoma of the contralateral breast: About 1% of the breast carcinoma patients may develop carcinoma in the another breast.
10. Ionizing Radiation is also a risk factor for developing of breast carcinoma, particularly if the exposure get during the development of mammary gland. .
11. Obesity: The obese ladies under 40 are at decreased risk of developing breast carcinoma because of anovulatory cycle in obese women and low progesterone level. Otherwise postmenopausal obese womens have increased risk of developing breast carcinoma.
12. Contraceptive: Various epidemiologic studies proved that use of contraceptive agent does not cause increased risk of developing breast carcinoma. Some studies show that a very low increase of

incidence may be possible among young ladies of long-term contraceptive users.[5]

13. Toxins: Several toxins like pesticide organochloride may cause breast carcinoma. It is believed that organochloride may have estrogenic effect in human body.
14. Exercise: Physical exercise is protective against breast carcinoma.

MALE BREAST CARCINOMA:

Carcinoma of male breast is very rare, accounting only 1% of the cases. Risk factors of male breast carcinoma are similar to the women's. First degree relatives affected by carcinoma breast are at high risk group. The other risk factors are age, exposure to exogenous estrogen, male infertility (for example klinefelter syndrome), obesity, gynaecomastia etc .

ANATOMY OF BREAST:

Morphologically the breast is a complex branching structure divided into multiple lobes . Each lobes is composed of 2 components: the terminal duct–lobular unit (TDLU) and the large duct system[6] the TDLU are the secretary unit of the gland and is formed by the lobules and terminal ductules. the duct system of the mammary gland contains

sub segmental duct, segmental duct, and finally collecting duct (lactiferous duct).

The large collecting ducts open into the surface of the nipple through 5 to 9 orifices. Numerous sebaceous glands also open independently of hair follicles. Ten to twenty. areolar protuberances called montgomery tubercles are formed by a collecting (lactiferous) duct associated with a sebaceous apparatus. These protuberances become prominent during pregnancy.[7]

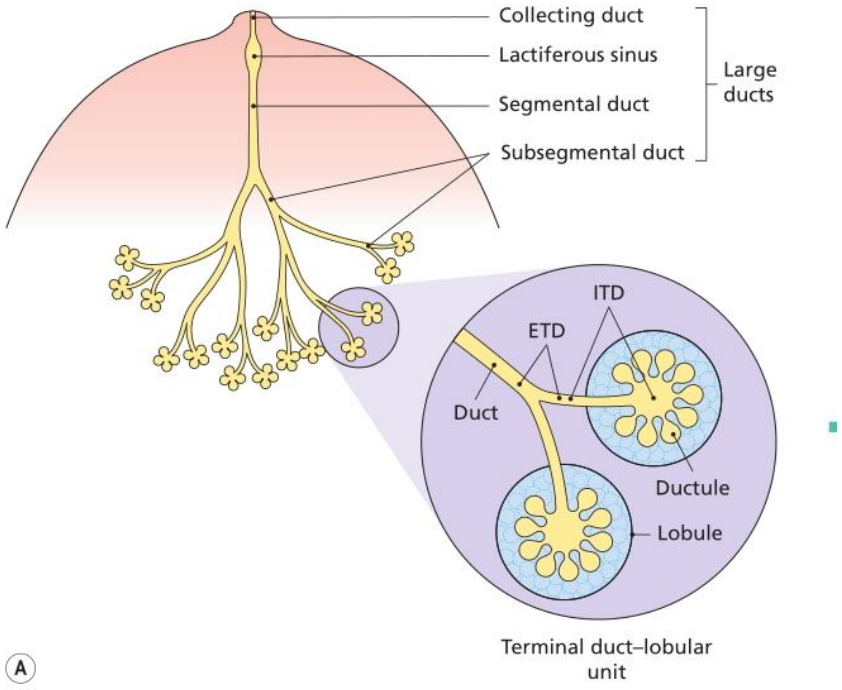


Fig: Diagram of breast morphology showing terminal duct–lobular unit (TDLU) subsegmental duct, segmental duct, and finally collecting duct (lactiferous duct)

GENOMICS OF BREAST CANCER

Total 5 to 10% of breast carcinomas are hereditary in origin. Some other studies show about twelve percent of breast cancers are familial.

From the literatures of recent studies, mutations of mainly two genes, namely BRCA1 and BRCA2, are responsible for familial breast cancer. the gene BRCA1 is located on chromosome 17q21, and BRCA2,is located on 13q12.3.

Several other genes also have been identified as cause of breast cancer development. Hereditary breast cancer may be associated with multiple cancer syndromes. Some common genes other than BRCA1,and BRCA2 are, fanconi anaemia (FANC) genes, CHK2, TP53 , and ATM tumour suppressor genes.[8]

For the development of breast carcinoma three major pathways have been proposed. First and most common pathway is germline mutation of BRCA 2 gene. These tumors are mostly the ER positive cases. The Mutation of BRCA 2 gene cause flat epithelial atypia then leads to atypical hyperplasia which is the precursor lesion of developing breast carcinoma.

Second pathway is germline mutation of gene TP53 gene and HER2 gene amplification leads to apocrine adenosis. Apocrine

adenosis is the precursor lesion for ductal carcinoma in situ, cases of HER2c positive breast carcinoma cases.

Third pathway is germline BRCA 1 mutation . In this group no precursor lesions have been established. These tumors are triple negative (ER negative, PR negative, and HER 2 negative) also termed as basal like .

DIAGNOSIS OF BREAST CANCER

Apart from clinical examination, many other methods are practiced for the detection and evaluation of breast disease. A combination of clinical examination, radiological and pathological examination is useful for diagnosis and management of breast cancer.

Mammography: Mammogram is one of the most suitable methods for screening of breast cancer. Screening of breast carcinoma with mammogram was introduced in the 1980s. By using mammography, extremely small tumors (1–2 mm) can also be detected in presence of calcification. Both the sensitivity and the specificity of mammographic breast screening is increased with age. In mammographic screening two principal findings are observed, the breast density and calcification. Carcinomas usually shows irregular dense areas. Clustered numerous, small, irregular calcified areas are

associated with malignancy. Mammographic findings are reported by using the Breast Imaging Reporting and Data System (BI-RADS) which is standardized by American College of Radiology (ACR) **.**[9]

Other radiological tests for detection of breast carcinoma are ultrasonography and Magnetic resonance imaging (MRI). Ultrasonography is particularly helpful in separating solid from cystic lesions. Magnetic resonance imaging (MRI) is also a useful and effective technique for the breast cancer detection, diagnosis, and staging. [10]

Cytology:

For cytological detection of breast malignancy two methods that have been used. FNAC (Fine needle aspiration cytology) is a method to obtain cytologic material from the lesion by aspirating with a fine needle. [11]

The Fine needle aspiration cytology procedure has many significant advantages is the low cost and the ability conclude a diagnosis within a very short period of time. As it is a very less time consuming procedure the treatment decisions can be made immediately by the clinicians.[97] Material obtained from FNAC also can be used for detection of hormone receptor (Estrogen receptor & progesterone receptor)**,**[12] oncoprotein expression.[449] and for kinetic studies.[13]

Another method of cytological test is imprint or aspiration of nipple secretion.

HISTOPATHOLOGICAL EXAMINATION:

The histopathological specimens of breast may be tur-cut biopsy. incisional biopsy or excisional biopsy, lumpectomy specimen or quadrantectomy or mastectomy (surgical removal of entire breast).[16]

Needle biopsy:

Biopsy is performed with large core needle for palpable masses (Tru-Cut) without any radiologic guidance. [16]

Open biopsy:

Open excisional biopsy from breast lesions is usually used to do for the tumor measuring 2.5 cm or less in diameter and for larger neoplasms incisional type of biopsy is practiced. Advantages of open biopsy is that it is a very highly accurate procedure; very low false-positive rate, (nearly zero), and false-negative rate (less than 1%). [14,15]

Incisional Biopsy:

Incisional biopsy of breast is very rarely practiced , almost always performed to evaluate unresectable malignant tumors of breast. Main aim of incisional biopsy is for confirmation of the clinical diagnosis and to know the ER, PR and HER2/neu status. [16]

Excisional Biopsy:

Complete removal of the lesion performed for the primary evaluation of a breast lesion. [16]

ANCILLARY STUDIES

For proper treatment of breast carcinoma the status of estrogen receptor (ER) and progesterone receptor (PR) status is also necessary. To know the ER and PR receptor status, Immunohistochemical (IHC) study is most commonly used technique in modern medicine.[17] The most important advantage of IHC marker study to know the hormone receptor status is it requires a very small amount of specimen.

MICROSCOPIC TYPES OF BREAST CARCINOMA

For morphological study of breast tissue two important points to be observed.

(1) Invasion into the stroma:-- based on the invasion into the stroma, the breast lesions are divided into in situ carcinoma and invasive carcinoma. In situ carcinoma is defined as if the tumor is confined to the epithelial component. If the tumor has invaded the stroma then it is termed as invasive carcinoma.

(2) Whether it is ductal or lobular type .[18]

IN SITU CARCINOMA

Ductal carcinoma in situ (DCIS):

DCIS represents tumors that the proliferated malignant epithelial cells limited to the ducts or lobules within the basement membrane layer . Variants of ductal carcinoma in situ are subdivided according to morphologic patterns, such as papillary, micropapillary, comedo-DCIS, solid pattern, cribriform, cystic and clinging.[19] Among these subtypes the Papillary variant of DCIS is believed, to be arised from larger ducts of breast and the other subtypes are, believed to originate from the terminal duct–lobular unit (TDLU).[20]

Comedo DCIS:

Charecterised by Pleomorphic cells with high grade nuclear morphology with central areas of necrosis.

Noncomedo DCIS:

In this pattern no central areas of necrosis or no high grade nuclei

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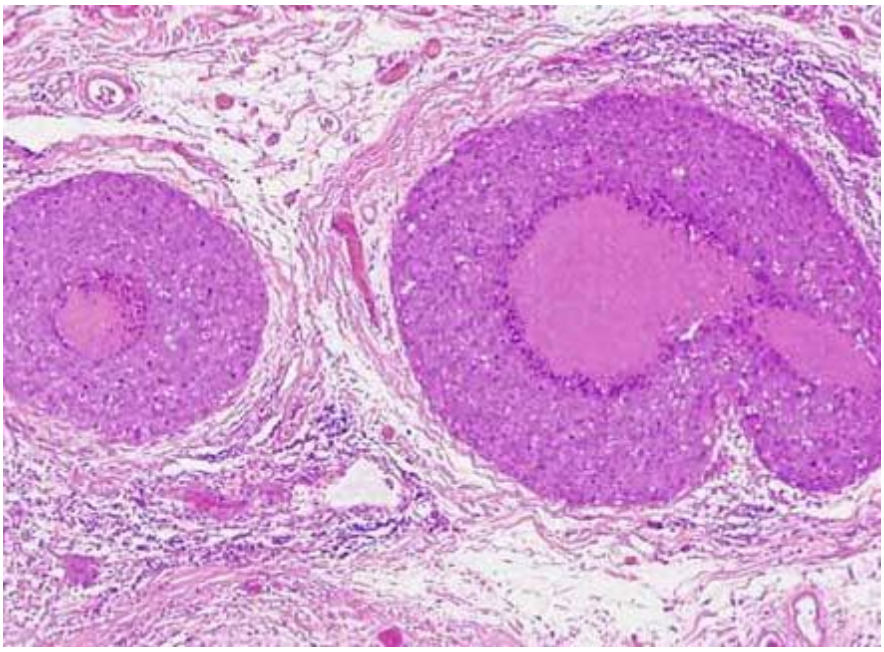


Fig: Comedo DCIS showing neoplastic cells within the basement membrane with central areas of necrosis.

Lobular carcinoma in situ (LCIS):

Lobular carcinoma in situ lesions are incidental finding in breasts removed for other reasons and they show similar features on gross examination. About 70% of cases of LCIS are multicentric [21] and 30–40% of LCIS are bilateral.[22]

Microscopic feature of the LCIS show distended lobules composed of relatively monomorphic , round to oval , small-to-medium-sized cells with round normochromatic nuclei. Sometimes the nuclei may be mild hyperchromatic[23]

Usually mitotic activity and necrosis are absent or very minimal.[24] Some LCIS cases are composed of medium to large size tumor cells exhibiting moderate to abundant cytoplasm with nuclear pleomorphism, and occasional prominent nucleoli. These lesions are referred as pleomorphic Lobular carcinoma in situ.[25]

INVASIVE CARCINOMA:

Tumors with detectable stromal invasion are termed as invasive carcinoma. Invasive carcinomas are divided in to sub categories based on morphologic variant and molecular characteristics'. On the basis of morphology, invasive carcinoma can also be divided into two major subtypes – ductal type and lobular type, like the in-situ lesions.

MAJOR HISTOLOGIC TYPES OF INVASIVE BREAST CARCINOMA:

IDC NST(Invasive ductal carcinoma no special type):

IDC-NST is the most common histopathological type of breast carcinoma. Approximately 70 % to 75 % of breast carcinoma cases are IDC-NST type. Microscopic, features are variable from case to case.

ILC (invasive lobular carcinoma):

It contributes 5 to 15 % of the total breast carcinoma cases. Microscopically, the tumour is composed of noncohesive cells with eccentrically placed nuclei arranged in Indian file and targetoid pattern. These tumors are mostly estrogen receptor (ER) and progesterone receptor (PR) positive but HER 2 negative.

Invasive Tubular carcinoma:

Total 5% of the invasive carcinomas are tubular carcinoma. Prognosis of this type is good because of low metastatic potential. Microscopically, tumor is composed of angulated tubules in haphazard arrangement lined by single layered of malignant epithelial cells eosinophilic to amphophilic cytoplasm with pleomorphic oval nuclei. Most of the tumors are Estrogen Receptor and Progesterone Receptor positive .

Invasive Mucinous carcinoma:

Another histological variant of breast carcinoma contributing approximately 2% to 3% of breast carcinoma cases. Grossly tumor is well circumscribed lobulated mass with gelatinous cut surface. Microscopically, neoplastic cells are seen floating in pools of extracellular mucin. Most of these cases are also estrogen receptor (ER) and progesterone receptor (PR) positive but HER2 negative.

Invasive Medullary Carcinoma:

Total Cases are about 1% to 2% of Breast carcinoma. Mostly seen in young women with well circumscribed mass with no calcification. Microscopically, syncytial growth pattern with pushing borders with higher nuclear grade. Lymphoplasmacytic infiltration is seen. These cases are mostly triple negative (ER, PR, HER2 negative).

Invasive micropapillary Carcinoma:

(1 % to 2%) Tumor clusters are seen within empty places with reverse polarity. Fibrovascular core is not seen. It expresses variable estrogen receptor (ER) and progesterone receptor (PR) and HER2 reactivity.

Metaplastic Carcinoma:

Incidence is less than 1% of the malignant breast tumors. Grossly the tumor is very large in size. Microscopically, tumors are composed of nonglandular (squamous type) and mesenchymal elements. These tumors are mostly triple negative . [99]

METASTASES OF BREAST CARCINOMA:

Carcinoma of Breast can spread by direct invasion, by hematogenous route or by the lymphatic route. [81]

Local invasion may spread normal breast parenchyma, nipple areolar complex, skin, underlying muscle, fascia, or to the chest wall.

Lymphatic spread: Most commonly involved lymph nodes for metastasis of breast carcinoma are the axillary N and the internal mammary LN, with the supraclavicular LN. [82]

From various studies it is expecting that about 40–50% of clinically detectable cases of breast carcinoma shows Axillary node metastases.[83] Supraclavicular lymph node with axillary lymph node involvement is present in approximately 20% of patients of the cases. Supraclavicular lymph node involvement is very rare in absence of negative axillary metastasis.[84] The internal mammary chain is the second most common group of lymph node drainage and it

contributes 22% of the metastatic lymph nodes of breast carcinoma. [85]

The common sites involving distant metastases are the bone, lung , liver, adrenal gland, ovary, CNS and eyes. [86,87,88] Metastasis to abdominal cavity, gastrointestinal tract [89,90,91] or rarely spleen may also occur. [92]

STAGING AND GRADING:

TNM staging system (T tumor; N, nodes; M metastases) is the most widely used clinical staging system for breast carcinoma.[26]

MICROSCOPIC GRADE:

Most widely used microscopic grading systems for invasive breast carcinoma is Bloom and Richardson grading system[27]. The Bloom and Richardson grading system is based mainly on architectural features. Another microscopic grading system Black grading system,[28]. based on the degree of nuclear atypia. Since from the prognostic point of view both architecture and cytology are important criteria, a joint grading system is proposed.[29,30]. The Nottingham modification of the Bloom–Richardson system includes the evaluation of mitotic activity along with the architectural features.[31] In this grading system, the grade is obtained by the sum of

the scores of three characteristic features, tubule formation, nuclear pleomorphism, and mitotic count. The total point after adding the scores 3 -5 regarded as final grade 1, Score 6 to 7 is grade II and score 8 to 9 is considered as grade III. [32]

MICROSCOPIC GRADING OF BREAST CARCINOMA (Nottingham modification of the Bloom–Richardson system)

Tubule formation :

1 point: Tubular formations in >75% of the tumor

2 points: Tubular formations in 10–75% of the tumor

3 points: Tubular formations in <10% of the tumor

Nuclear pleomorphism :

1 point: Nuclei with minimal variation in size and shape.

2 points: Nuclei with moderate variation in size and shape.

3 points: Nuclei with marked variation in size and shape.

Mitotic count:

1 point:-- mitosis 0 to 9

2 points: -- Mitosis 10 to 19

3 points – mitosis more than 20.

SUM OF POINTS	FINAL GRADE
3–5	I
6–7	II
8–9	III

The total point is the sum of the three points. In this system Grade I (point 3 to 5) is considered as well differentiated, Grade 2 (point 6 to 7) Moderately differentiated and Grade 3 (point 8 to 9) poorly differentiated.[93]

PROGNOSIS

The prognosis of carcinoma breast depends upon multiple number of clinicopathological factors.[33–34]

Age:

Breast carcinoma patients aged less than 50 years at the time of diagnosis show excellent prognosis. Prognosis tends to decline gradually with age after the age group of 50 years.[35] Some recent studies have shown that prognosis among young ladies (=35 years) is also not good as the older ladies.[36]

Invasiveness:

Invasiveness is a very important prognostic factor for carcinoma breast. In situ carcinomas may be curable with mastectomy because of less probability of metastasis.[37] Sometimes comedocarcinoma type DCIS can associate with metastases which is a sign of poor prognosis.. The carcinomas invading to the underlying skeletal muscle are difficult to do surgery.

Early diagnosis:

Early detection of cancer is a good prognostic factor.

Size:

Higher diameter of the tumor have higher chances of nodal metastases and lower survival rate. It is established that microscopic size determination has a greater prognostic predictive value than gross size of the tumor. If an invasive tumor is having both in situ and invasive component, the size of the invasive component will be more important prognostic predictor than the gross size of the tumor**.** [38]

Cytoarchitectural type:

Prognosis of both invasive ductal and lobular carcinoma are similar.[39] Some morphologic types of ductal carcinoma shows good

prognosis. Tubular ca, cribriform ca, medullary ca (when strictly defined), pure mucinous ca, papillary ca, adenoid cystic ca, and secretory (juvenile) ca are the variants with good prognostic outcome.[40,41,42] Signet ring carcinoma is associated with very bad prognosis.

Margins:

Tumors with infiltrating margins have a bad prognosis than tumors with pushing 'margins.[43,44]

Tumor necrosis :

Necrosis is associated with tumors of high histological grade and increased incidence of lymph node metastases.[45] So tumor necrosis is a sign of bad prognosis.

Inflammatory carcinoma:

In breast carcinoma cases the signs of breast erythema and skin thickening are poor prognostic indicators. Most of these patients show evidence of distant metastasis.

Stromal reaction:

Breast carcinoma with an absence of inflammatory reaction at periphery have a lesser chance of nodal metastases and hence these tumors show good prognosis.[46]

Microvascular density (MVD):

Invasive breast carcinomas with higher amount of vascular component are tend to be more aggressive in nature and so the prognosis will be poor.**[47]**

Fibrotic focus:

Tumors composed of a fibrotic area is a sign of hypoxia and lymphangiogenesis and it is unfavorable for good prognosis.[48]

Skin invasion:

Breast carcinomas with overlying skin invasion is have a poor prognosis.**[49]**

Nipple invasion:

Nipple involvement with the malignant tumor have higher incidence of metastases to the axillary lymph nodes**.[50]**

Tumor emboli:

Tumor emboli are divided mainly into two types, blood vessel emboli and lymph vessel tumor emboli. Malignant tumors of breast having blood vessel tumor emboli show poor prognostic outcome.[51] Similarly tumor emboli inside lymph vessels is also associated with high recurrence rate.[52] Intravascular tumor emboli containing apoptotic body and mitotic figure is a sign of bad prognosis.[53]

Microscopic grade:

The Nottingham modification of the Bloom–Richardson system is used for microscopic grading of breast carcinoma. Scores 3 -5 regarded as grade 1, Score 6 to 7 is grade II and score 8 to 9 is considered as grade III.

Estrogen receptors (ER):

Several studies established that ER-positive tumors have a longer survival rate than the ER negative tumors.[54] Estrogen receptor (ER) and progesterone receptor (PR) positive tumors show good response to hormonal therapy.

HER2/*neu*:

Tumors with over expression of **HER2/*neu*** show good response to trastuzumab but a poor response to chemotherapy**.**[55]

BCL2:

Patients with BCL2 positive tumors show long-term survival rate**.**[56] Some recent studies shows a correlation between BCL2 positivity and ER positivity in breast carcinoma.[57]

BRCA1 status:

From previous studies it is thought that breast carcinomas with *BRCA1* mutation carriers a poor survival rate, without receiving adjuvant therapy**,**[59] but some recent studies show that breast cancer patients with either *BRCA1 or BRCA2* mutations have same prognosis as the non-carriers. [58]

Cell proliferation:

Cell proliferation index is a very important prognostic indicator,[60] particularly for the cases with metastasizing to lymph nodes. It is determined by mitotic count**,**[61] by Ki-67 or analogous immunostaining**.**[62] High proliferative index tumors show poor prognostic outcome.

Metastases to axillary lymph node:

Axillary LN metastasis is an important prognostic parameter for breast carcinoma.[63] Positivity of the axillary lymph node status indicates poor prognosis. During histological study of nodal status of carcinoma breast, the level of involvement either low, or high[64] the number of node involved,[65] the amount of the tumor component in the node,[66] status of extra-nodal spread ,etc should be examined thoroughly.[67]

Distant metastasis:

Presence of distant metastasis is a very poor prognostic indicator.

HORMONE RECEPTORS

Both estrogen receptor (ER) and progesterone receptor (PR) are located in the nucleus of the cells. The Hormone estrogen and progesterone are transported to the nucleus and forms hormone – receptor complex. It is believed that the ER and PR receptors regulate some genes which control growth of cells. Therefore Estrogen receptor status is important in breast cancer for therapeutic target to inhibit the growth of tumor that influenced by estrogen.

Estrogen Receptor was discovered in the 1960s. From several studies it is established that ER status is effective to manage the growth of the ER positive tumors by endocrine targeting inhibition of the receptor. The inhibition of ER can be done such by selective ER modulators therapy or by aromatase inhibitors or oophorectomy etc.[68]

The status of hormone estrogen and progesterone receptors positivity is a very important indicator for hormone therapy and chemotherapy in breast carcinoma cases. Among these two, the estrogen receptor(ER) positivity status is considered as a very useful marker to predict the response to hormonal therapy or chemotherapeutic management of breast carcinoma[69]

These hormone ER and PR receptor status is measured by immunohistochemical staining method. Other methods to detect the hormone receptor are dextran-coated charcoal & sucrose gradient assay.[70], situ hybridization technique and by PCR.[71]

In immunohistochemical study two parameters are evaluated, first one the number of tumor cell nuclei stained with the marker ,and secondly the intensity of the reaction.

HER 2 NEU

Human epidermal growth factor (HER 2)gene in malignant breast tumor was discovered in 1987. The gene was originally termed as *NEU* because it was first recognized in neuroblastoma / glioblastoma tumor cell lines of rat.[68] Approximately 15% to 20% of breast cancer cases show HER2 positivity and it is an indicator of poor prognostic outcome of chemotherapy and hormonal therapy.[68]

The breast cancer patients with positive HER2 NEU immunohistochemistry have worse prognosis in absence of adjuvant systemic therapy. It is also established from Several studies that *HER2*-neu overexpressing tumors have relative resistance to endocrine therapy such as tamoxifen,[72]

Many other organs like ovary, uterus, stomach, colon, urinary bladder, prostatic gland, salivary gland etc also show over expression of HER 2 NEU gene.

The prognostic importance of HER 2 NEU positivity in breast carcinoma is variable among several studies.

Even some studies (Slamon DJ, Clark GM, Wong SG) shows that, HER-2/neu expression status have more prognostic importance than other prognostic factors like ER, PR receptor positivity status in lymph node-positive breast cancer cases.[73]

Some other studies found HER-2 neu expression in breast carcinoma have inverse prognostic relationship with both ER and PR positivity status. According to these studies HER 2 neu positive cases have relative resistance to endocrine therapy.[74]

Several clinical trials show that it is effective to use anti–HER-2 agents therapy in patients with HER-2–expressing breast cancer.[75,76]

Grading of the immunohistochemical staining for HER2-neu

STAINING PATTERN	SCORE	ASSESSMENT
No staining / Or membrane staining in < 10% of the tumor cells	0	Negative
A faint membrane staining more than 10% of the tumor cells.	1+	Negative
A weak to moderate complete membrane staining in more than 10% of the tumor cells	2+	Weakly positive
A strong complete membrane staining is more than 30% of the tumor cells	3+	Strongly positive

MOLECULAR SUBTYPES OF BREAST CANCER BASED ON ER, PR AND HER 2 NEU STATUS:

Along with the clinical behavior and histopathological classification of malignant tumors of breast, the molecular classification is also very important to access prognosis and for appropriate therapy . The major molecular subtypes of Ca breast cancer determined by gene expression of ER , PR and HER2**.**[80]

In this classification carcinoma breast cases are divided into 4 major subclasses, Luminal A, Luminal B, HER2/neu and Basal-like[a] [32]

Luminal A -

ER and/or PR positive, HER2 negative. (most common subtype. 50% of invasive breast cancers are Luminal A type).

Most of the older women and men patients belong to this group.

Luminal B -

ER and/or PR positive, HER2 positive/ negative. (Second most common type ~20% of invasive breast cancers).

HER2/neu -

ER negative PR negative, HER 2 positive. (~15% of invasive breast cancers). Approximately 50% of patients of HER 2 positive group shows ER positivity. Most common patients in this group are young ladies.

Basal like or triple negative breast cancers -

ER negative, PR negative, HER 2 negative.(~15% of invasive breast cancers)

GATA 3

The GATA family of transcription factor consists of 6 transcription factors (GATA-1 to GATA-6) characterized by ability to bind with the "GATA" sequence of DNA . GATA transcription factor regulates differentiation of various tissue types, including mammary gland, T lymphocytes, kidney, nervous system and hair follicle. Although GATA 3 is expressed in many tissues, but it is not always detectable by immunohistochemical staining due to its low level in the tissue. [77]

Therefore, some publications say that mutation of genes encoding GATA family of transcrtption factors may have role in the development of various types of cancers in human body. [78]

Many malignant tumors of GIT, ovary, lung, and brain show altered expression of GATA 4, GATA 5, and GATA 6. [78] Various studies established that GATA 3 is a high specific marker for breast carcinoma and carcinoma of urothelium.[77]

GATA 3 expression in breast carcinoma is established from several studies but few important points are still not clear about the clinical use of immunehistochemical study of GATA 3.

1. Several studies suggest that GATA3 is expressed more commonly in ER positive tumors than ER-negative tumors.[79] But GATA 3 expression in triple negative (ER,PR, HER2

negative cases) tumor is most relevant to prove whether the tumor is from mammary origin or not. [77]

2. Positivity of GATA 3 in metaplastic (sarcomatoid) carcinoma is not studied to distinguish it from other spindle cell malignancies in the breast.
3. Clinically GATA 3 can be used as a marker to diagnose the metastatic tumors originating from breast. [77]

MATERIALS AND METHODS

This is a study of both prospective and retrospective type of data analysis of patients diagnosed as invasive breast carcinoma in Institute of Pathology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai. Our study period was 3 years (June 2015 to may 2018).

For our study, we randomly selected 50 cases of invasive breast carcinoma with already known ER , PR and HER 2 NEU status . The slides are prepared from formalin fixed paraffin embedded archived tissue blocks of tru-cut biopsy and mastectomy specimens. The biopsies were processed for light microscopy with routine haematoxylin and eosin stain. Immunohistochemistry was done as per standard protocol.

IMMUNOHISTOCHEMICAL EVALUATION:

Immunohistochemical analysis of GATA 3 was performed in paraffin embedded tissue blocks by using polymer HRP system. The thickness of the sections were 4 micron prepared (cut) with microtome from formalin fixed paraffin embedded tissue block samples and transferred onto positive charged slides. Heat induced antigen retrieval was done as per procedure . The antigen was bound

with monoclonal antibody (Pathnsitu) against GATA 3 protein, then detected by adding secondary antibody.

ANTIGEN	VENDOR	SPECIES (CLONE)	DILUTION	POSITIVE CONTROL
GATA 3	PATHNSITU	Mouse monoclonal	Ready to use	Urothelial Carcinoma

INCLUSION CRITERIA:

Breast carcinomas with known ER,PR and HER 2 NEU status in tru-cut biopsy and mastectomy specimens.

EXCLUSION CRITERIA:

1. Benign lesions of Breast.
2. Insufficient representative material.
3. Breast carcinoma cases with unknown ER, PR and HER 2 NEU status.
4. Lack of paraffin blocks with representative tumor tissue was excluded from the study population

METHOD OF DATA COLLECTION

The clinical data are collected from surgical pathology records of Institute of Pathology, Madras Medical College, Chennai. Age, Sex, size of the tumor, surgical procedure, gross findings of the tumor, microscopic findings on hematoxylin and eosin (H&E) stained histopathological examination, status of the estrogen receptor (ER) ,progesterone receptor (PR) and HER-2 immunohistochemistry reaction are collected from both the registers of routine biopsy and immunohistochemistry record.

INTERPRETATION AND SCORING SYSTEM:

The slides immunohistochemically stained with GATA 3 marker were analyzed for the positivity or negativity of the antibody reaction, staining pattern, percentage of cells that stained with the reaction and intensity of the reaction. Any intensity with more than 5% of cells showing positive staining for GATA3 is considered as positive .

Scoring:

GATA3 is a nuclear marker and its reactivity is scored as 0 to 4 + according to the percentage of cells stained. The extent of nuclear reactivity is graded as follows -

Score 0 = (0- 5%) less than or equal to 5% of positively staining tumor cell considered as negative.

Score 1+ = 6% to 25 % cells positive.

Score 2+ = 26% to 50% cells Positive.

Score 3+ = 51% to 75% cells Positive.

Score 4+ = More than 75 % cells are positive.

Intensity:

Intensity of staining is recorded as weak, moderate or strong.

STATISTICAL ANALYSIS

The statistical evaluation was performed with Microsoft office excel software system. Initial data from the histopathology register is collected and compared with the findings of the GATA 3 immunohistochemical analysis of the cases. The GATA 3 expression was compared and correlated with clinicopathological data like age, sex, size of the tumor, lymph node status etc. Pearson Chi square test was also used to analyze these data. In our study, the P value less than 0.05 is considered significant.

OBSERVATION AND RESULTS

During our study period from June 2015 to May 2018 (3 years) we studied the malignant tumors of breast both in Prospective and retrospectively. We studied total 50 specimens of Carcinoma breast diagnosed by histopathological examination in the Institute of Pathology, Madras Medical College and Rajiv Gandhi Govt General Hospital- Chennai. The specimens were selected randomly from the formalin fixed archived tissue blocks. The status of ER, PR and HER 2 neu reactivity is already known for all these cases. Out of these 50 cases 45 specimens obtained from MRM (Modified Radical Mastectomy), one case breast conservative surgery, one excision Biopsy, two tru-cut biopsy and one wide local excision specimen.

Procedure	Number of specimen
MRM (Modified Radical Mastectomy)	45
Breast conservative surgery	1
Wide Local Excision	1
Excision Biopsy	1
Tru-cut biopsy	2

GENDER:

Out of the 50 cases, 49 were female patients and one male. The patient was 55 years male histologyically diagnosed as invasive breast carcinoma – no special type , Grade II.

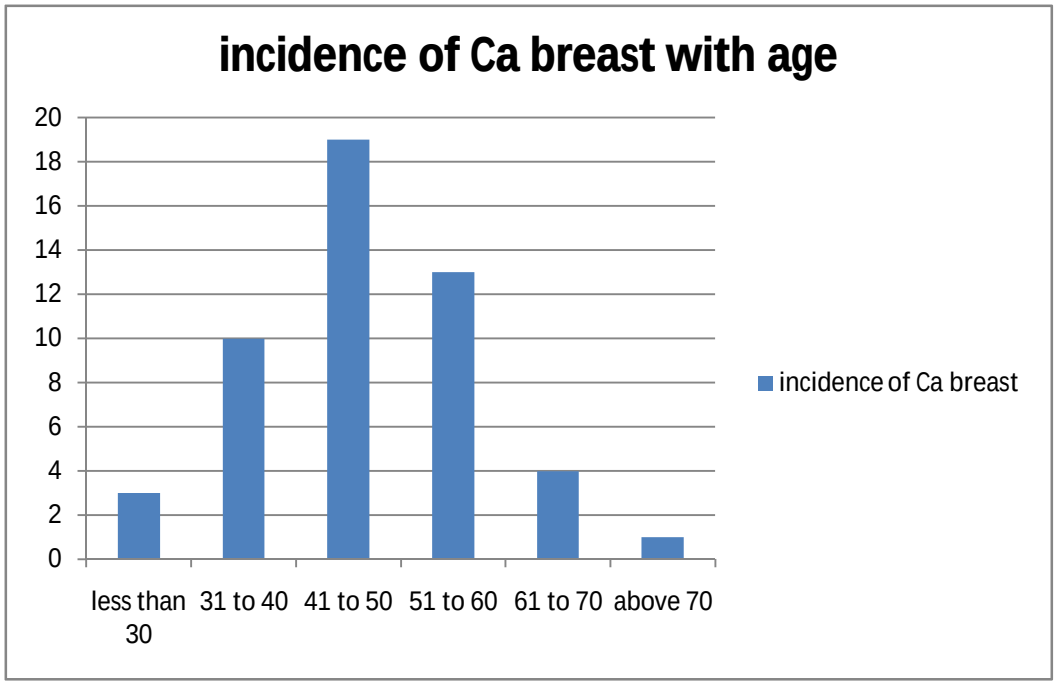
SEX	**NUMBER OF CASES**
Female	49
Male	1

AGE WISE INCIDENCE OF BREAST CARCINOMA:

In this study, age of the patient was divided into 6 groups as less than 30 years, 31-40 years, 41-50, 51-60, 61-70 and above 70 years. Age wise incidence of breast carcinoma cases were as follows.

Age group	Number of Cases
Less than 30	3
31 to 40	10
41 to 50	19
51 to 60	13
61 to 70	4
71 +	1

Chart: incidence of breast carcinoma in relation to age.



In our study the peak incidence of breast carcinoma is 41 to 50 years of age. Carcinoma of breast is rare below the age of 30 years.

GATA 3 SCORING :

Among these 50 cases of breast carcinomas, total 36 cases (72%) are GATA 3 positive and 14 Cases (28%) GATA 3 negative. Out of 36 positively stained, 6 cases score 1+, 5 cases score 2+, 11 cases score 3+ and 14 cases score 4+ .

Chart: GATA3 positive cases 72%, negative 28%

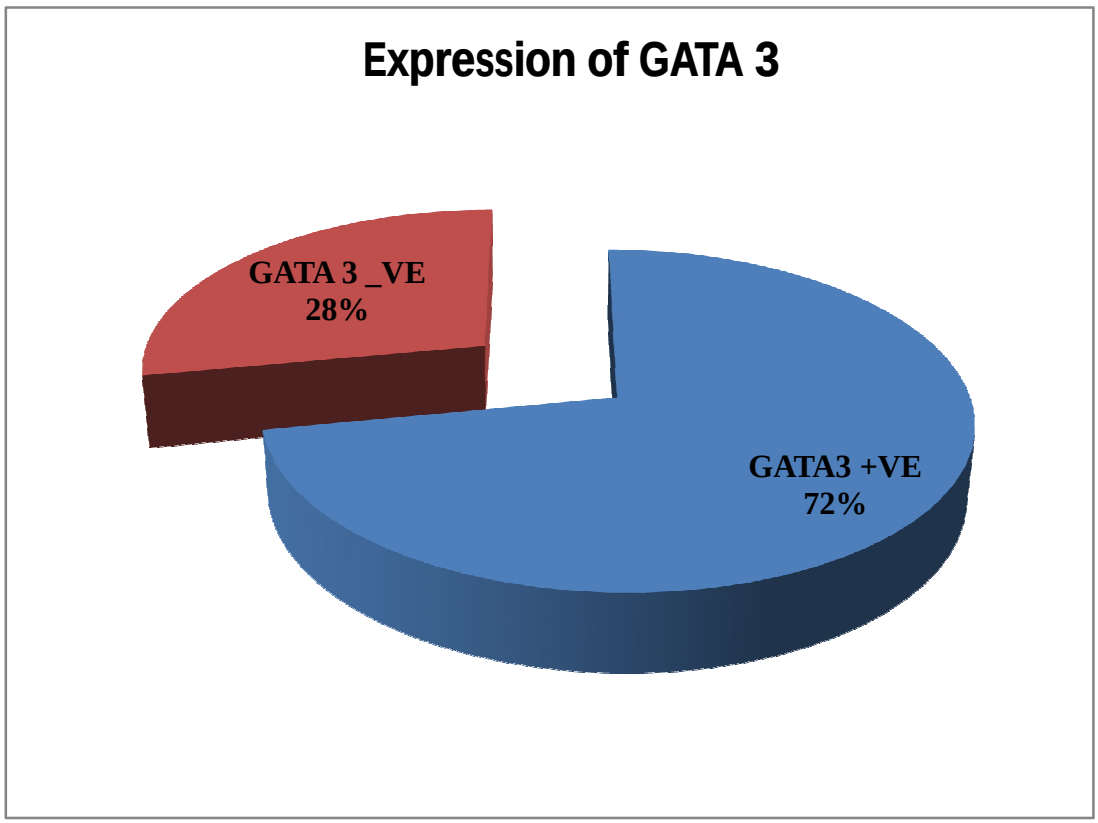
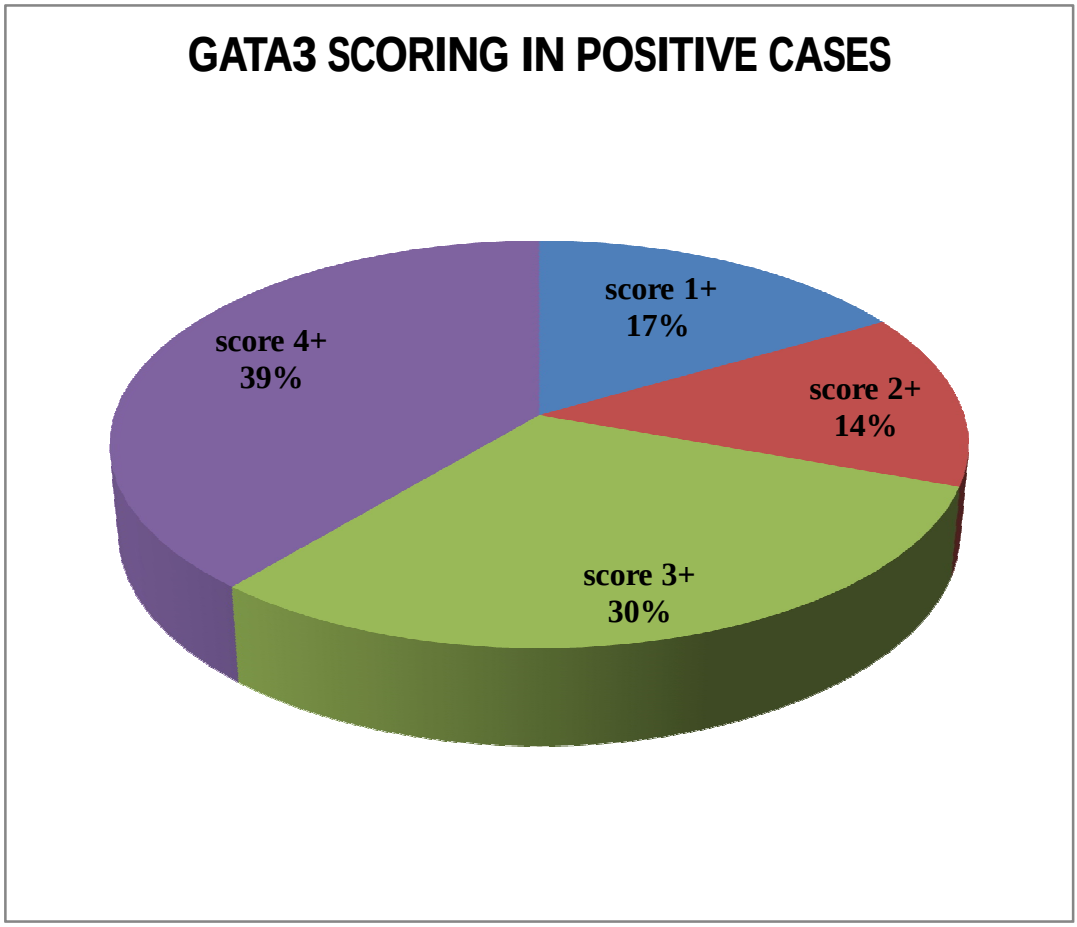


Chart : GATA3 positive cases with scoring, 1+, 2+, 3+ and 4+ cases

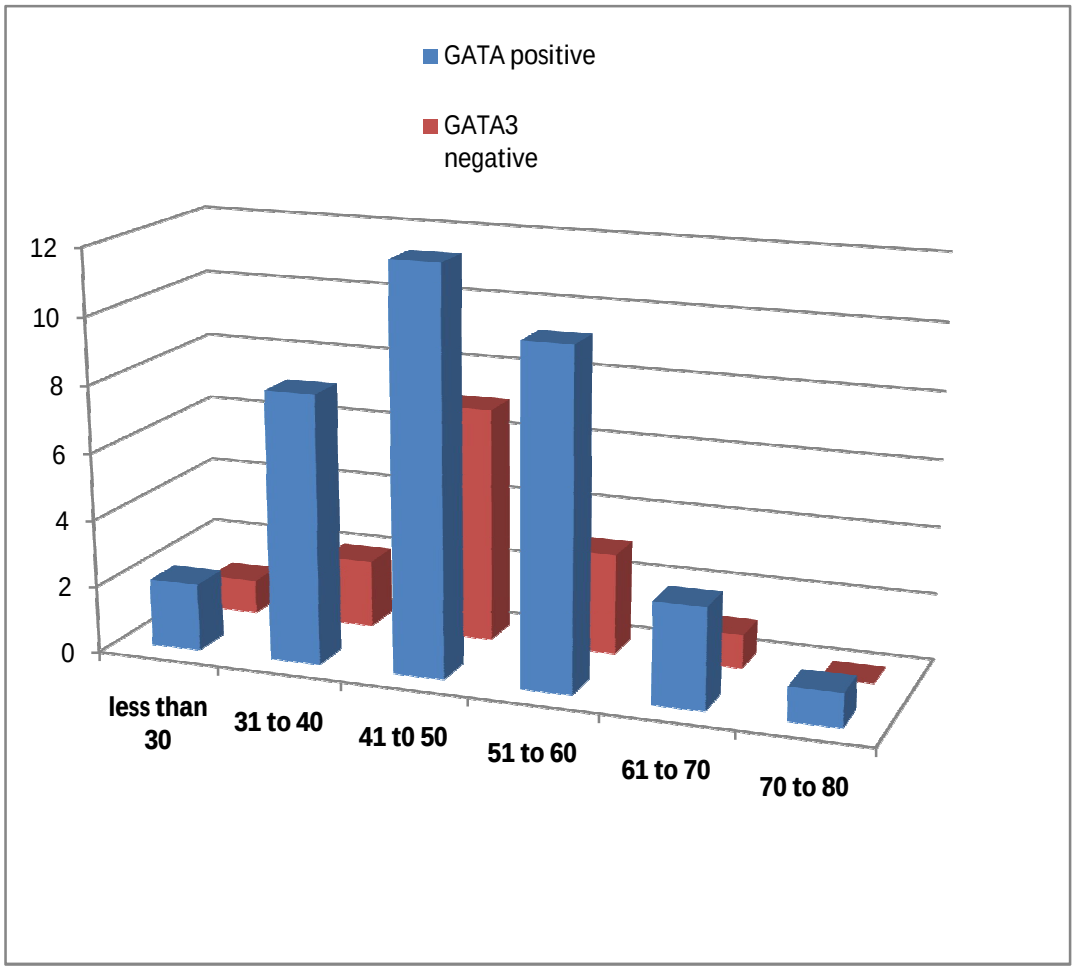


GATA3 expression with AGE

In this study, under 30 years, out of 3 cases 2 showed positivity to GATA 3. For the age 31 to 40 years, total 8 cases of out of total 10 . For the age group 41 to 50, total 12 were GATA3 positive out of 19 cases. For 51 to 60 (10 GATA3 positive out of 13), 61 to 70 (5 positive out of 4) and above 70 years 1 positive out of one.

Age	GATA3 positive	GATA3 negative
<30	2	1
31-40	8	2
41-50	12	7
51-60	10	3
61-70	3	1
70-80	1	0

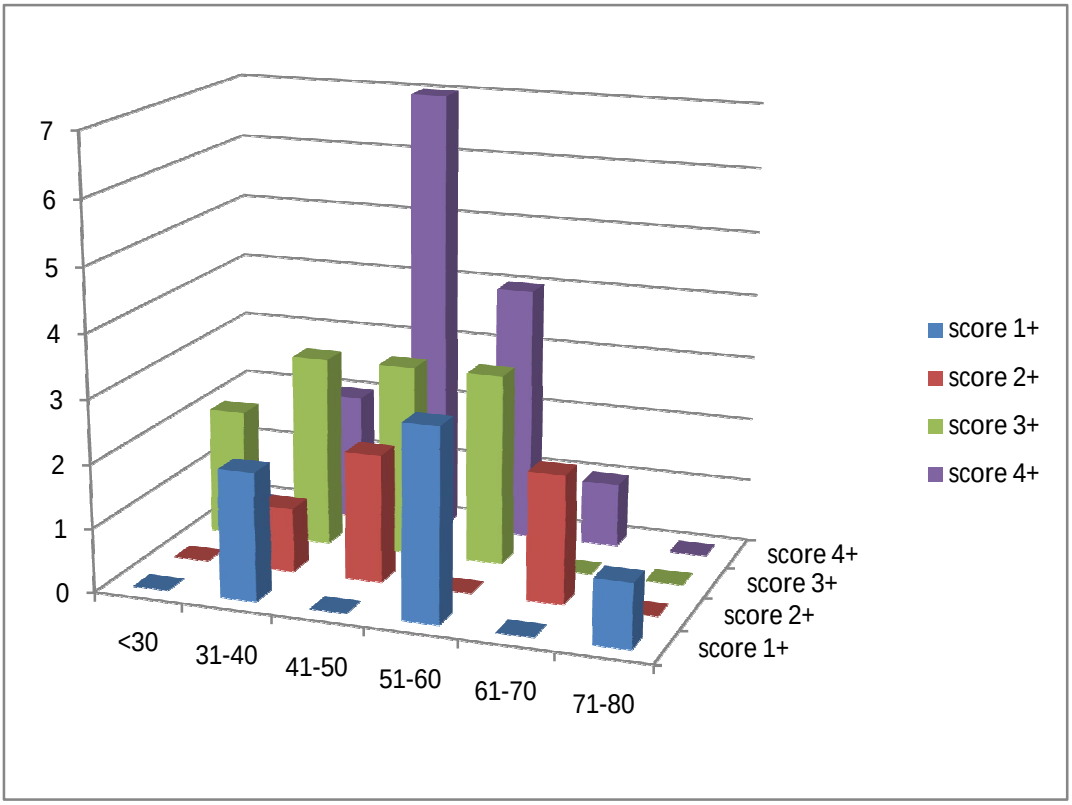
Chart: showing correlation of GATA3 expression and age:



GATA3 SCORING WITH AGE

Among the positive cases of GATA3 , number of cases with scoring is as follows.

Age	Score 1+	Score 2+	Score 3+	Score 4+
<30 years	0	0	2	0
31 to 40 years	2	1	3	2
41 to 50	0	2	3	7
51 to 60	3	0	3	4
61 to 70	0	2	0	1
71 to 80	1	0	0	0

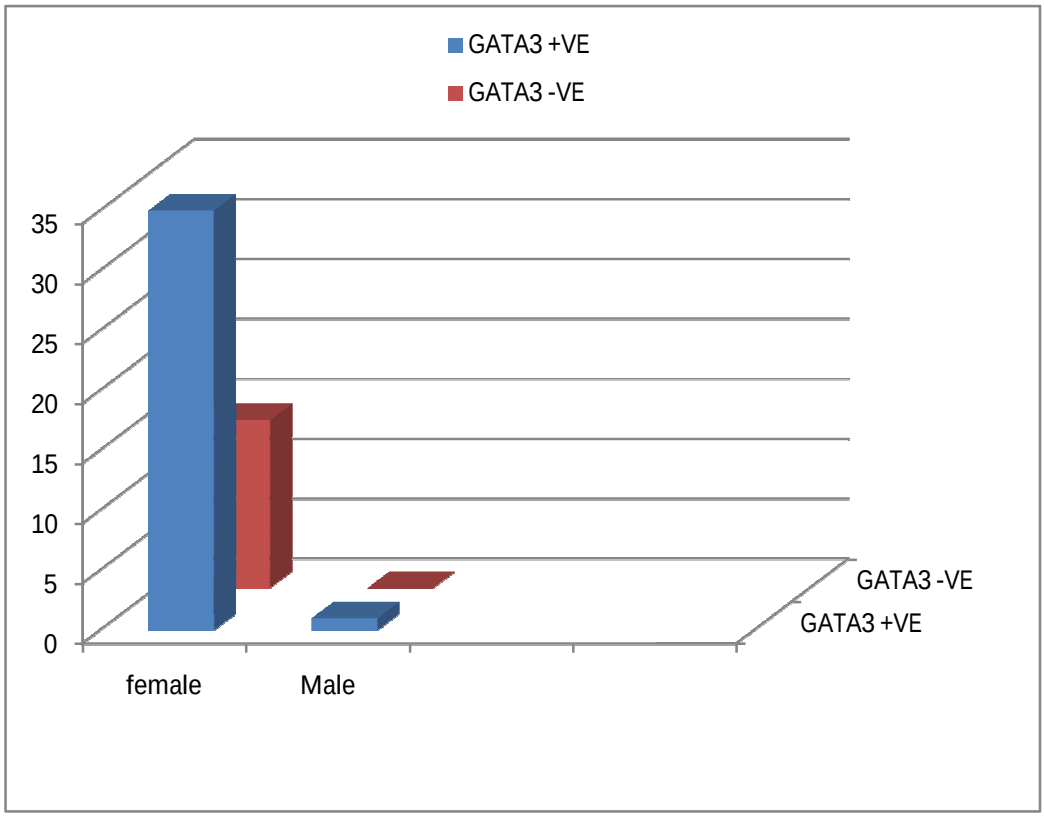


GATA 3 expression with Gender :

Out of 50 cases number of female patient was 49, and number of male patient was one.

Sex	Positive	Negative
Female	35	14
Male	1	0

Chart : GATA3 expression with Gender.



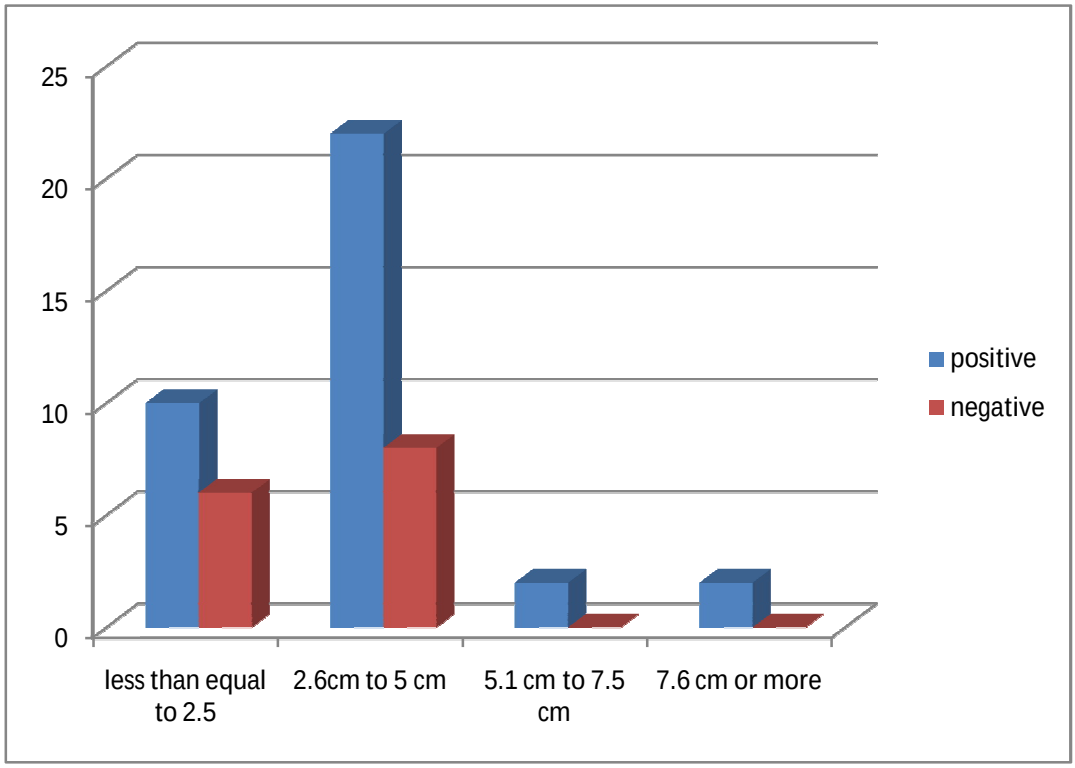
From this study, we observed that immunohistochemical expression of GATA 3 in breast carcinoma was not affected by gender.

GATA3 EXPRESSION WITH TUMOR SIZE (MAXIMUM DIAMETER):

We divided the gross size of the tumors in four groups. First group is the tumors less than equal to 2.5 cm in maximum diameter ,second group 2.6 to 5 cm, third 5.1 to 7.5 cm and forth group 7.6 cm or more.

Size of the tumor	GATA 3 positive	GATA 3 negative
less than or equal 2.5	10	6
2.6 to 5 cm	22	8
5.1 to 7.5 cm	2	0
7.6 cm or more	2	0

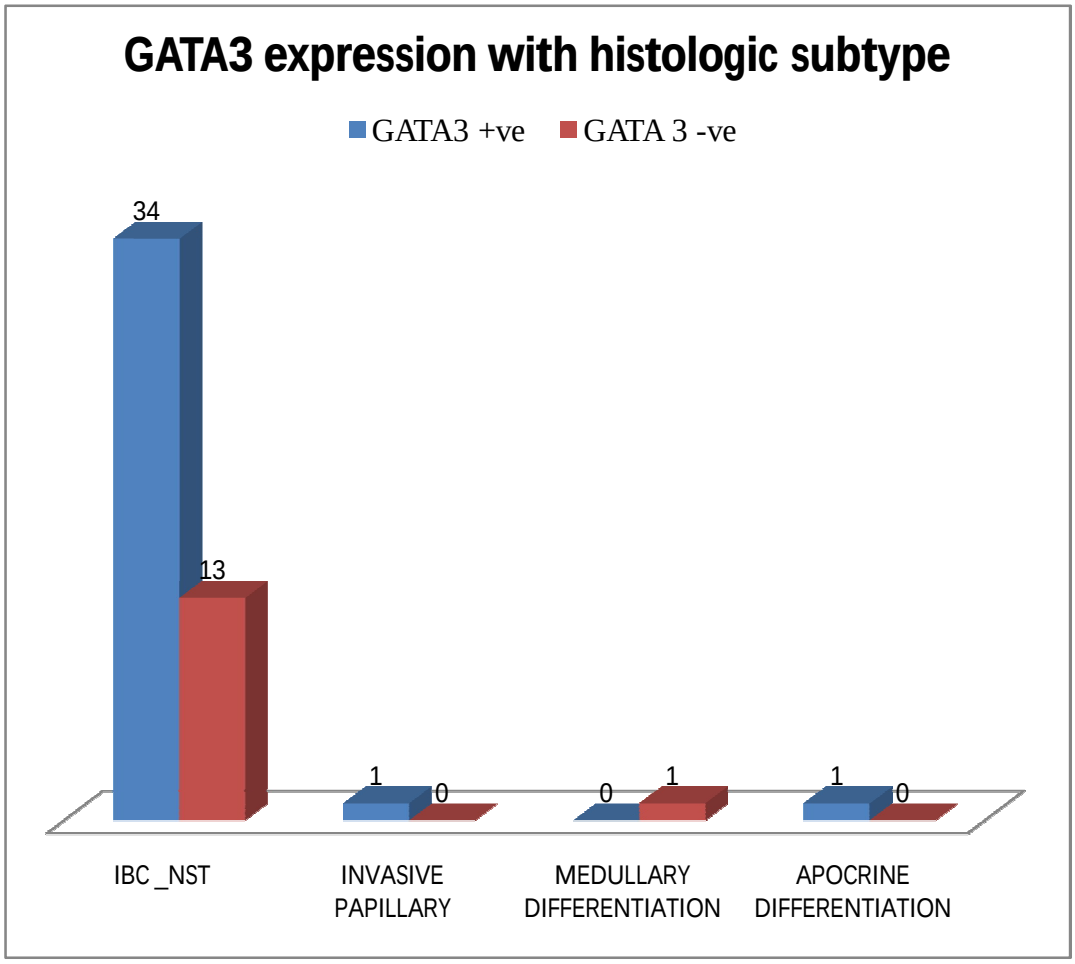
Chart: GATA3 expression with tumor size.



GATA 3 EXPRESSION WITH MICROSCOPIC VARIANT :

In our study majority of the cases were invasive breast carcinoma, no special type (IBC-NST) contributing 47 cases out of total 50 cases. Other variants were papillary variant, medullary differentiation, and apocrine differentiation representing one case from each.

HISTOLOGIC TYPE	GATA3 POSITIVE CASE	GATA3 NEGATIVE CASES
IBC – NST	34	13
Invasive Papillary Type	1	0
Medullary Differentiation	0	1
Apocrine Differentiation	1	0



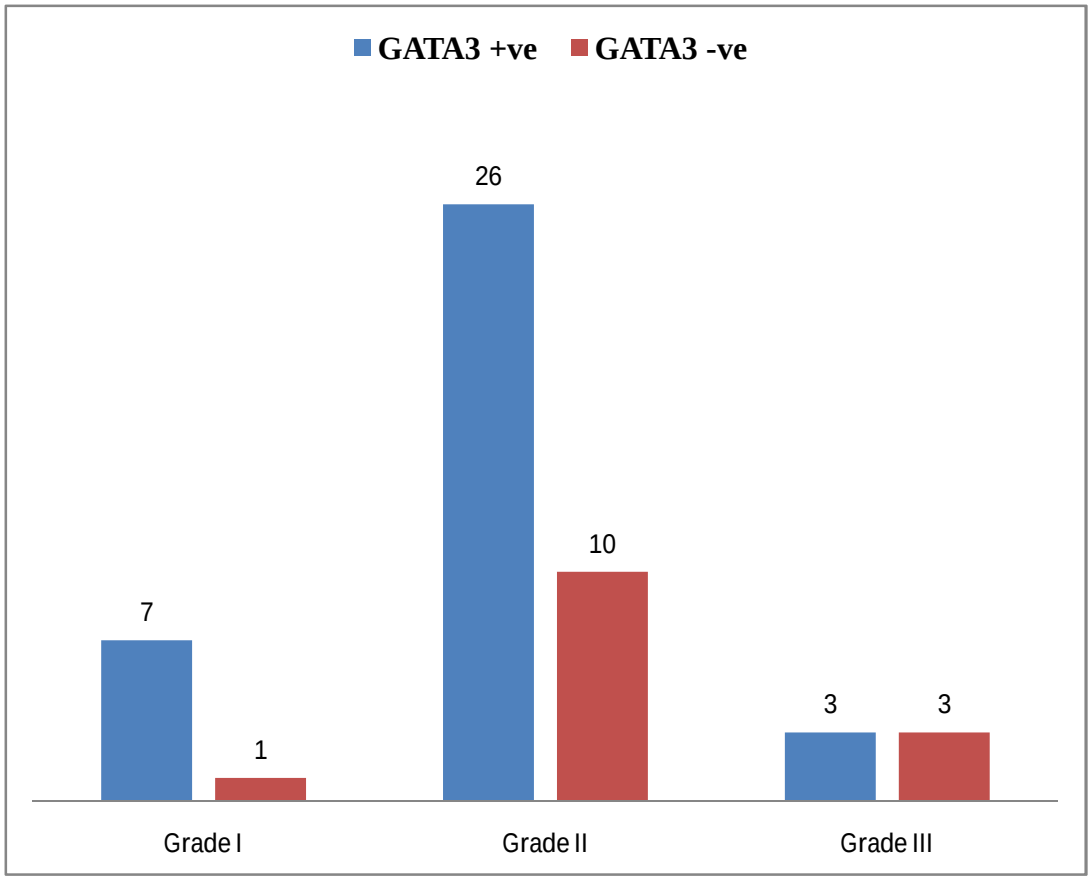
From the study we got the evidence that majority of the IBC-NST (invasive breast carcinoma no special) type shows reactivity to GATA 3 marker. Here we got 72.34% cases of IBC- NST are GATA 3 positive. Invasive papillary and apocrine variant also shows positivity to GATA 3. The medullary differentiation case did not show reaction to the marker.

GATA 3 EXPRESSION WITH MODIFIED BLOOM RICHERDSON GRADING SYSTEM FOR BREAST CARCINOMA:

From the histopathological data and the microscopic examination of H & E slides the population of histological grade were Grade I (total 8 case) , of grade II (total 36 cases) and Grade III (total 6 cases).

Histologic grade	**GATA 3 +ve**	**GATA3 –ve**
Grade I	7	1
Grade II	26	10
Grade III	3	3

Chart GATA 3 expression with the histological grade of the tumors:



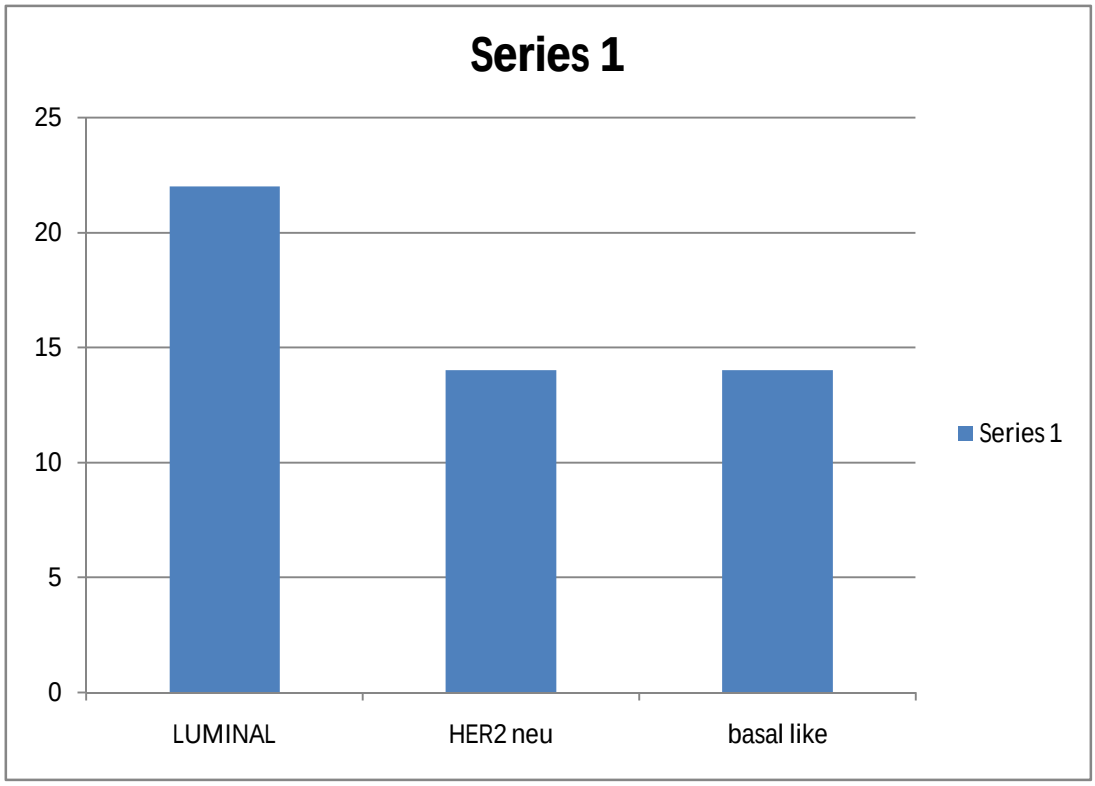
From our study we observed that the GATA 3 is expressed in all the 3 grades of Modified Bloom Richardson grading system. Our study concludes that the expression of GATA 3 is independent of histological grade of the tumour.

GATA 3 EXPRESSION WITH MOLECULAR SUBTYPES IN OUR STUDY BASED ON ER, PR AND HER 2 NEU STATUS:

In our study the the number of molecular subtypes as follows.

Subtype	ER , PR, HER 2 status	Number of cases
Luminal	ER +ve and/or PR+ve, HER2 +ve/-ve	22
HER2/neu	ER PR -ve or(-ve) , HER 2 positive	14
Basal-like	ER (-ve) ,PR(-ve) HER 2 (-ve)	14

Chart: Molecular subtypes of breast ca cases in our study

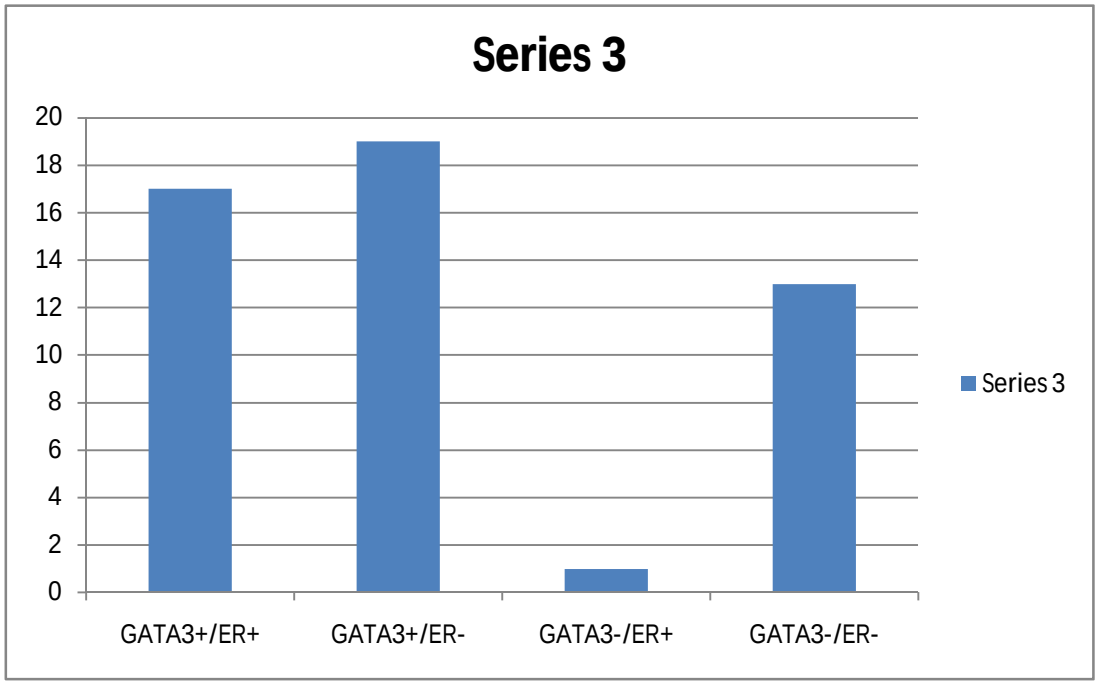


GATA3 expression with ER status.

The correlation between GATA3 and ER in our study is observed as follows.

GATA3	ER	NUMBER OF CASES
Positive (+)	Positive (+)	17
Positive (+)	Negative (-)	19
Negative (-)	Positive (+)	1
Negative (-)	Negative (-)	13

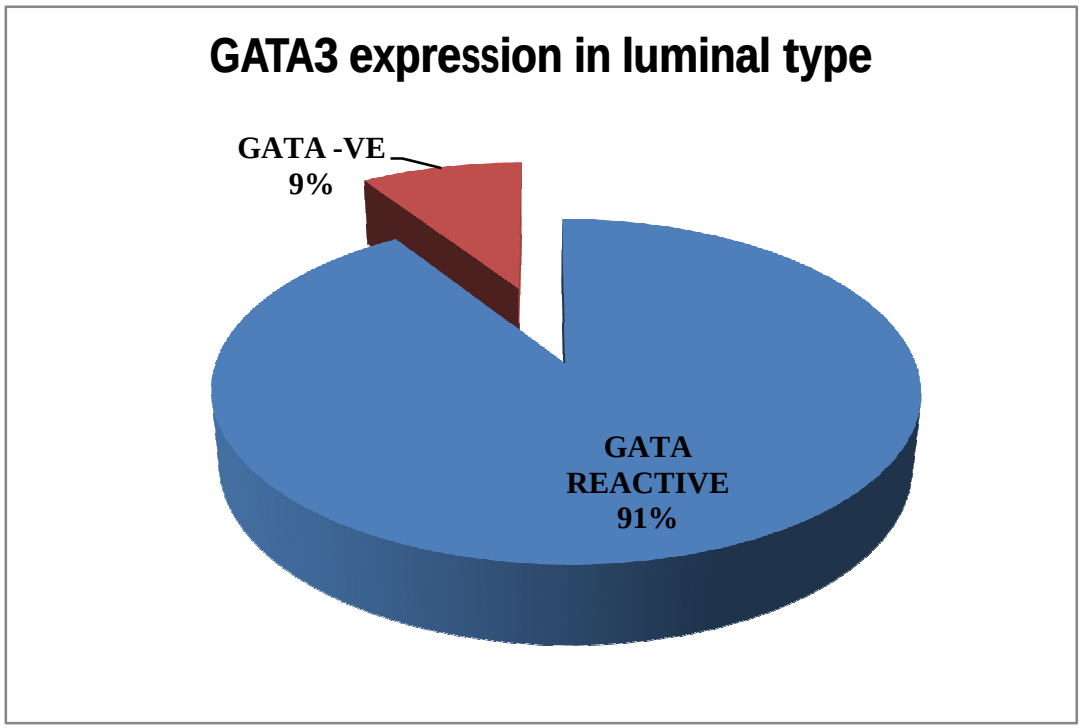
Chart : GATA3 expression in relation with ER



GATA 3 expression with luminal type:

In our study total 22 cases were luminat type . Out of these, 20 show GATA3 expression and 2 case show non- reactive to GATA3.

Chart: GATA3 expression with luminal type cases.



GATA3 positive scoring in luminal type:

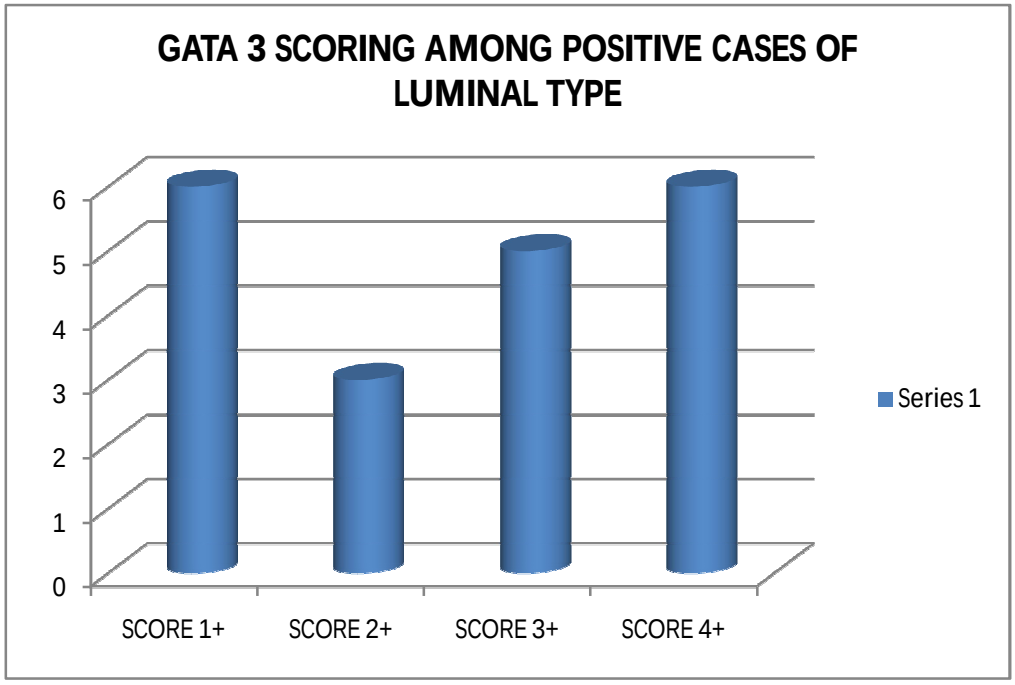
The GATA 3 scoring among the luminal type cases are. As mentioned before we considered Score 0 or negative when the positively staining tumor cell less than or equal to 5% of the total tumor cells.

Score 1+ = 6% to 25 % cells positive. Score 2+ = 26% to 50% cells Positive .

Score 3+ = 51% to 75% cells Positive. Score 4+ = More than 75 % cells are positive

	NUMBER OF CASES
SCORE 1+	6
SCORE 2+	3
SCORE 3+	5
SCORE 4+	6

Chart: The GATA 3 scoring among the luminal type



From this study we found that most of the luminal type of the tumors express GATA3 .It was evident that luminal tumors can express any score of among the GATA 3 positive cases.

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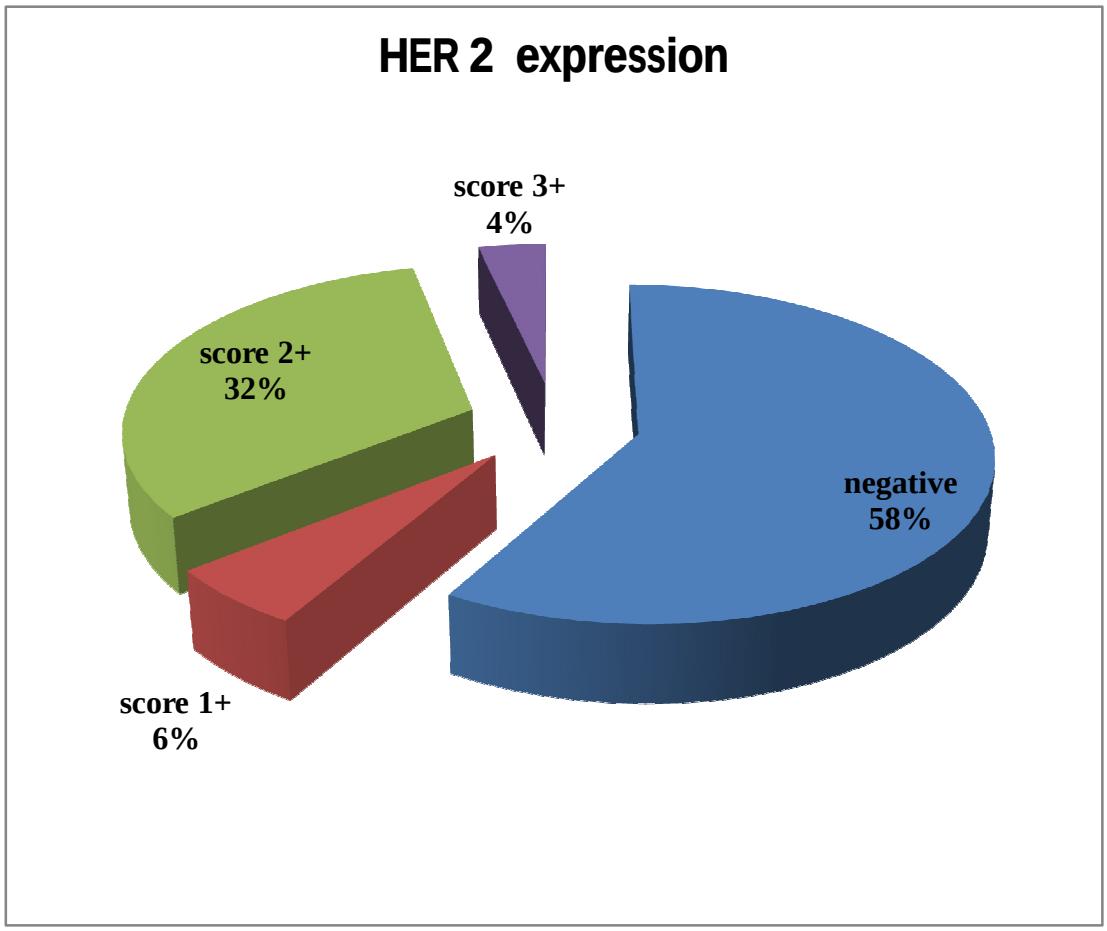
IMMUNOHISTOCHEMICAL EXPRESSION WITH HER 2 NEU :

In this study total 30 (60%) cases are HER 2 neu positive and 20 (40%) cases are HER 2 neu negative. Out of these positive cases 2 showed 1+ score, 11 showed 2+, and 17 showed 3+.

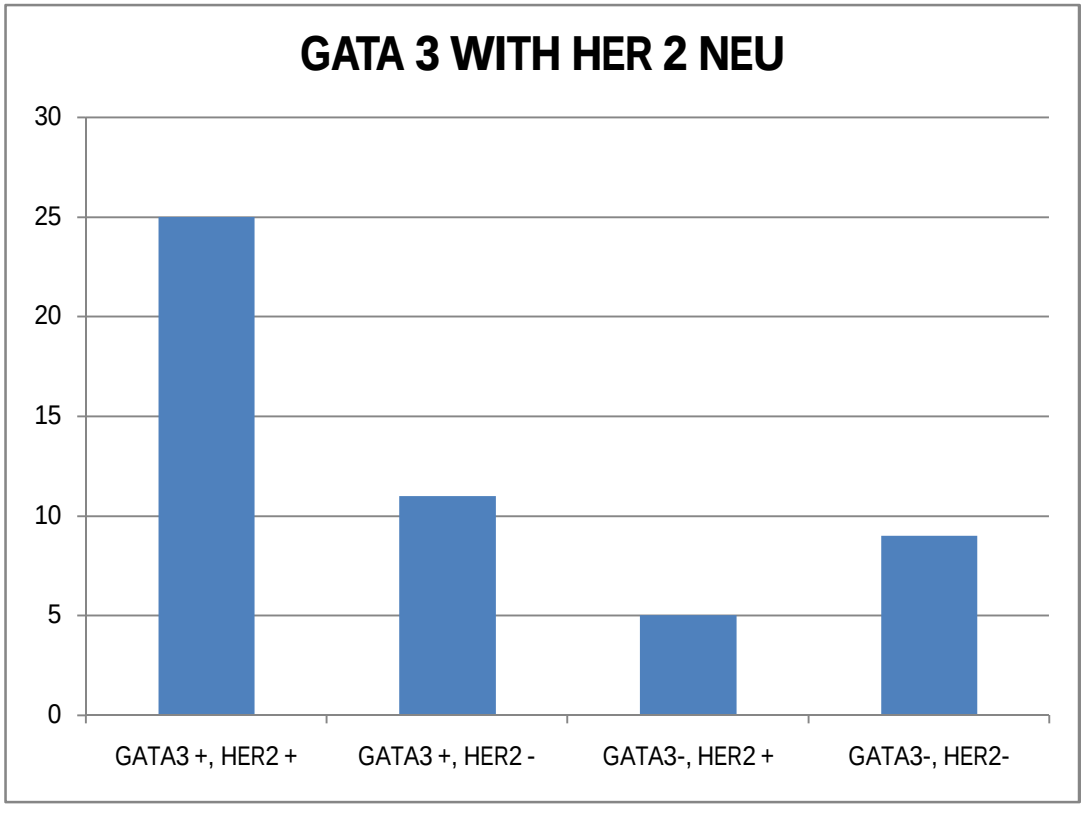
Chart: HER 2 neu expression



EXPRESSION OF GATA 3 WITH HER 2 NEU EXPRESSION.

The GATA3 expression in relation with HER2 neu is as follows.

	CASES
GATA3 +VE, HER2 +VE	25
GATA3 +VE, HER2 -VE	11
GATA3 -VE, HER2 +VE	5
GATA3 -VE, HER2 -VE	9



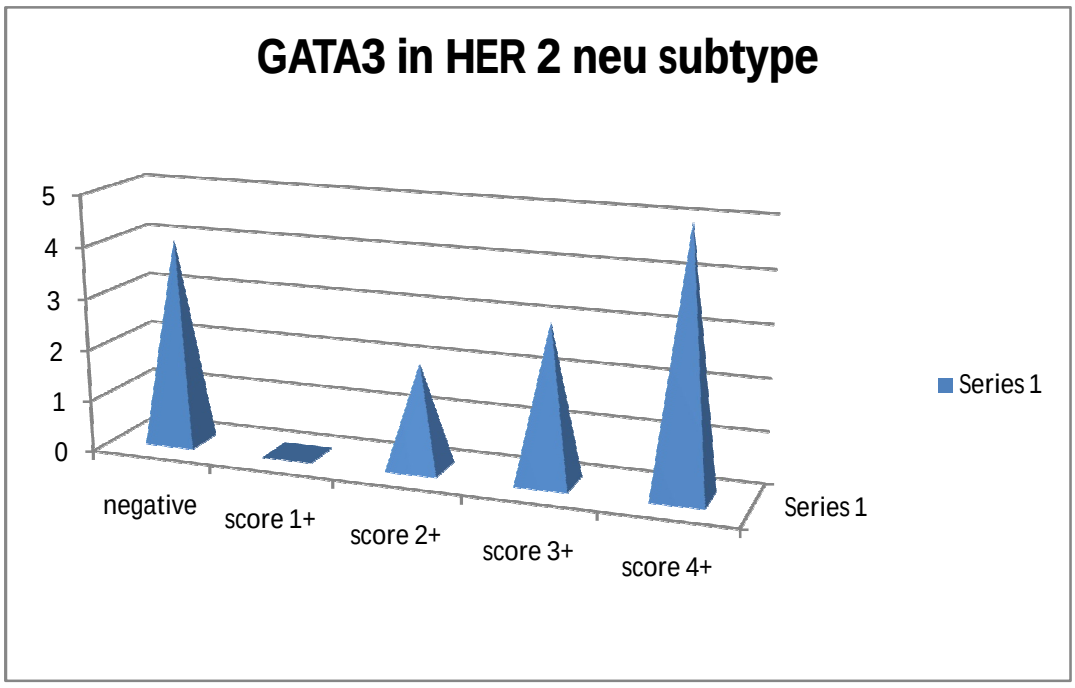
From our study we observed that expression of GATA3 is independent to HER 2 neu expression.

GATA 3 EXPRESSION WITH HER 2 SUBTYPE OF BREAST CARCINOMA:

In our study total 14 numbers (28%) of cases were HER 2 subtype. This subtype composed of Positive HER2 but ER expression may or may not be present. Among the 14 cases, 4 cases were GATA 3 negative and 10 cases were GATA3 positive.

GATA3 scoring	Number of cases of HER 2 subtype
Negative	4
Score 1+	0
Score 2+	2
Score 3+	3
Score 4+	5

Chart : GATA3 expression with HRE 2 subtype



From the study we observed that degree of expression of GATA 3 is not significantly correlated with the expression of HER 2 neu.

GATA 3 EXPRESSION IN (BASAL LIKE)TRIPLE NEGATIVE CASES

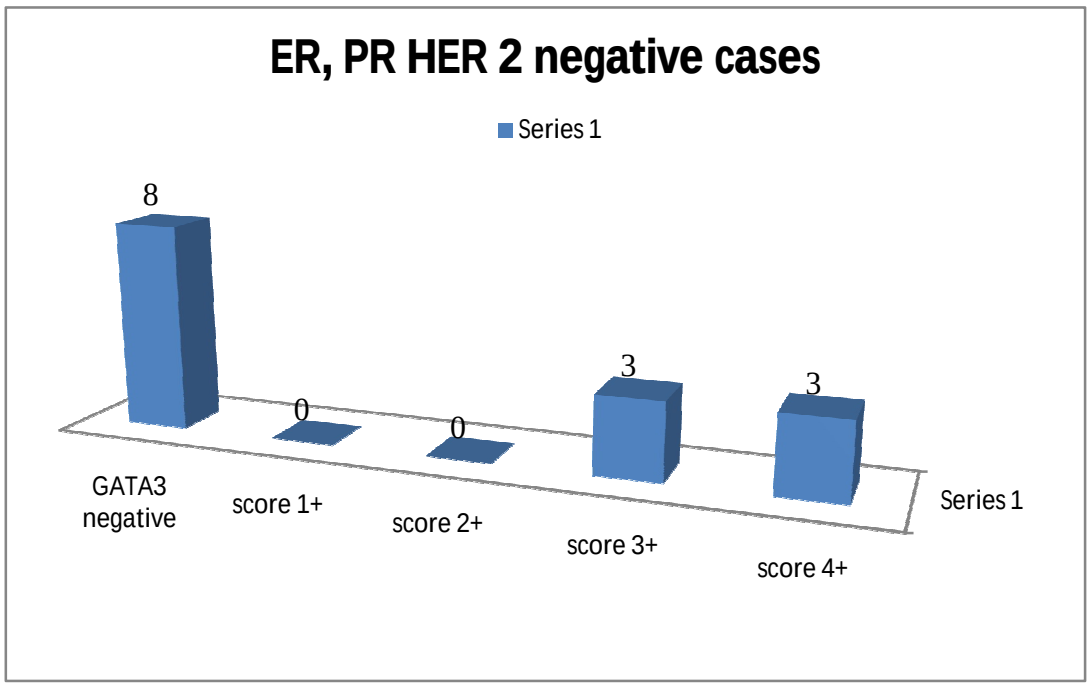
Among these cases total 14 cases were found to be non reactive to ER, PR and HER 2 neu.(BASAL LIKE). Out of these 8 of the triple negative cases show non reactive to GATA3 also. None of the cases shown GATA3 score 1+ and 2+. The remaining 3 positive cases shows GATA3 score 3+ and another 3(21.5%) cases score 4+.

GATA 3 Negative among triple negative cases =8 (57%)

Number of cases GATA 3 Scoring 1+ and 2+ nil.

Positive scoring 3+ cases 3 (21.5%) and 4+ case = 3 (21.5%)

Chart : GATA3 Expression in Triple Negative Cases



It was found that majority (57%) of the triple negative (basal like) cases do not express GATA3.

COLOUR PLATES

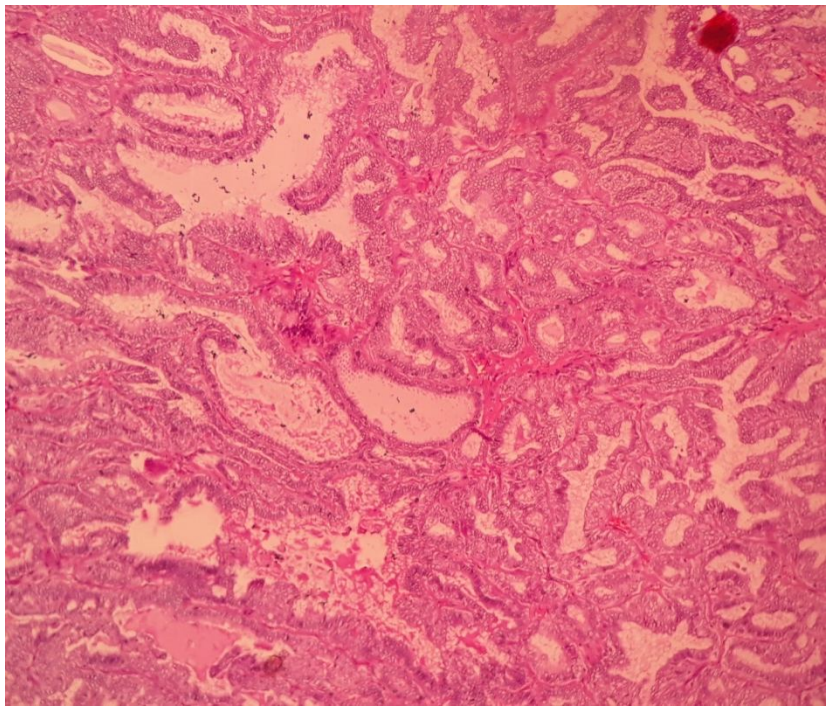
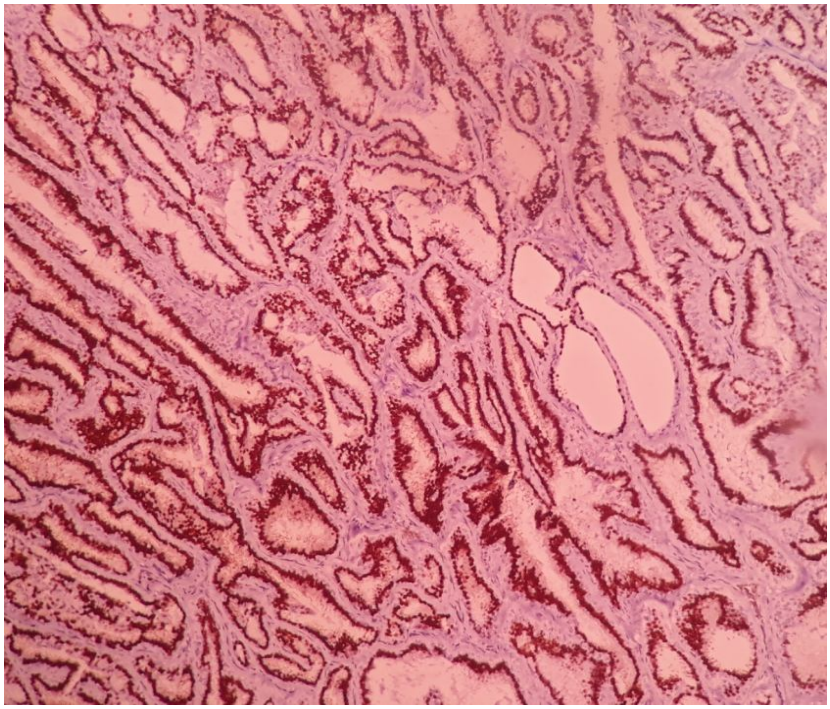


FIG: invasive breast carcinoma, papillary variant



IHC for Estrogen Receptor: 90% positive (5+3)

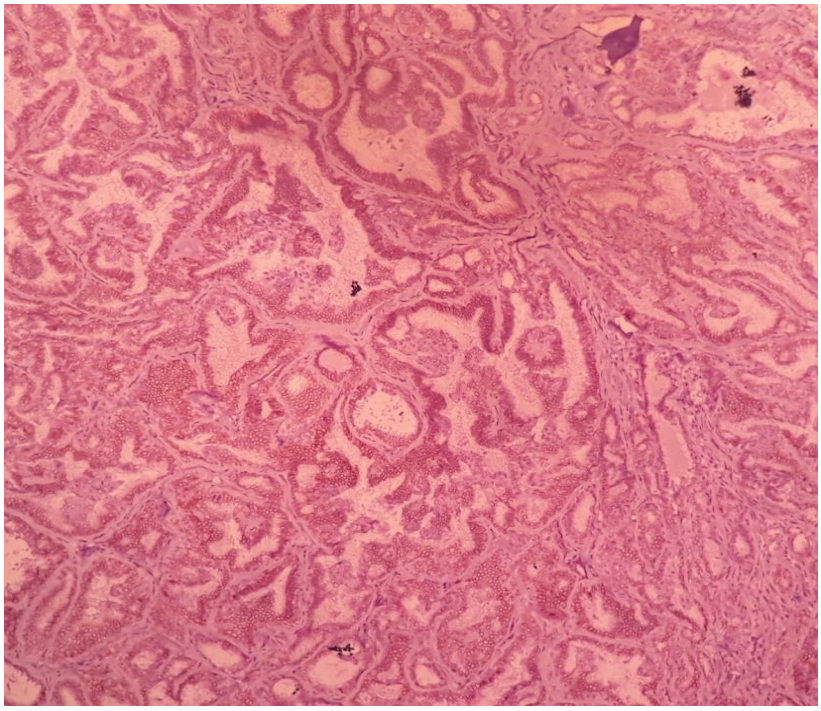
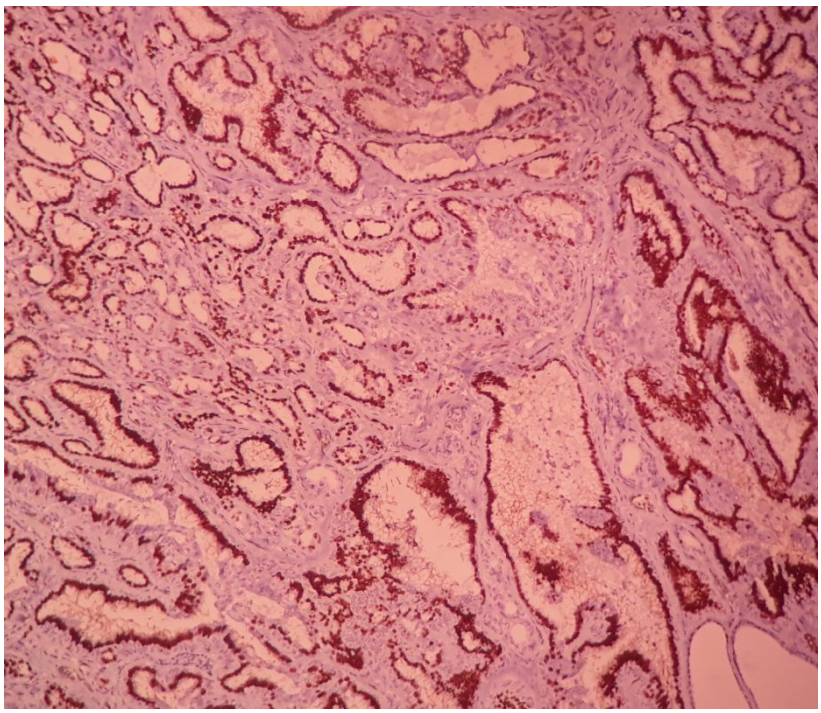
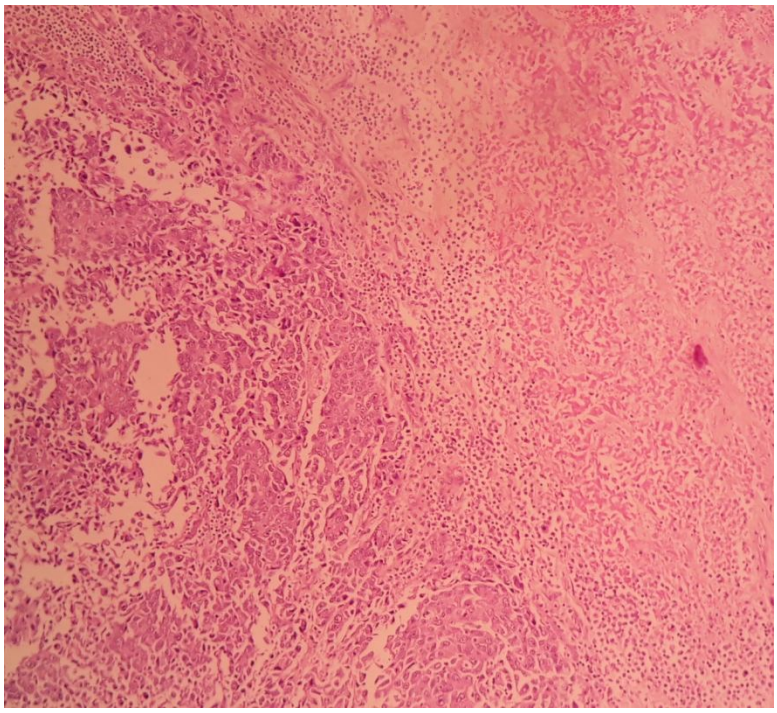


Photo: IHC for Progesteron Receptor (PR)- 90% positive (5+3)

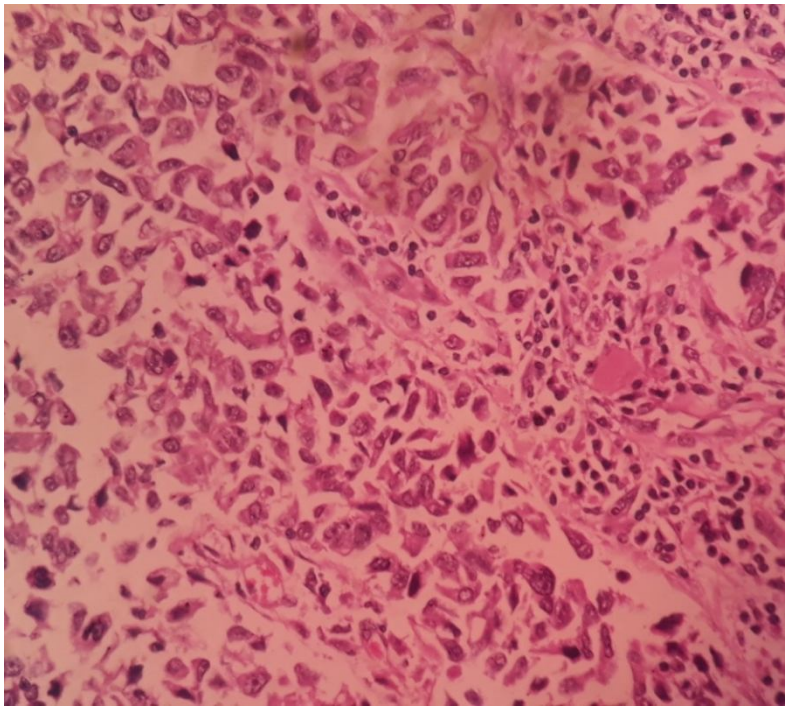


HER 2: showing positive staining (3+)

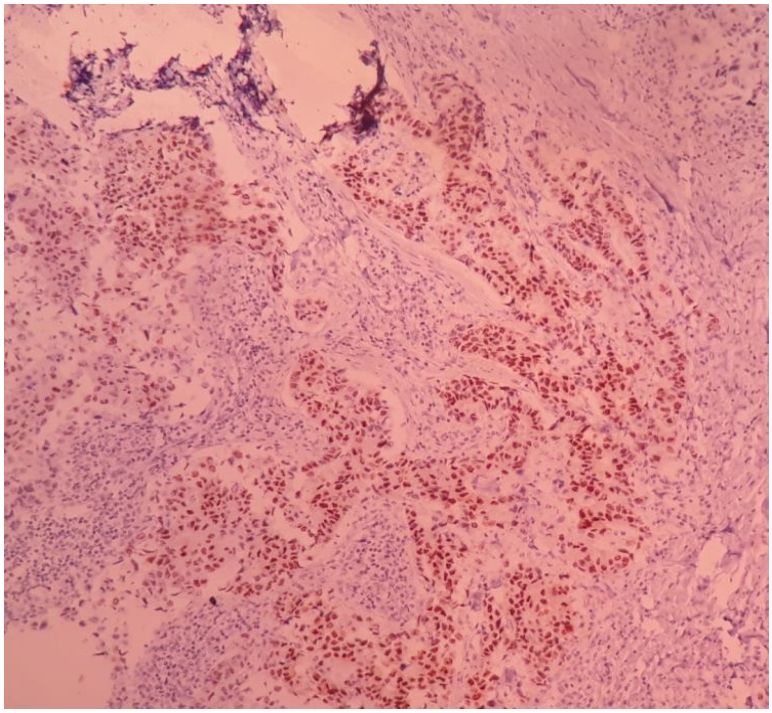
Case : Invasive Breast Carcinoma, No special type:- Sheets of Malignant epithelial cells with eosinophilic cytoplasm, Pleomorphic hyperchromatic nuclei, with areas of necrosis.



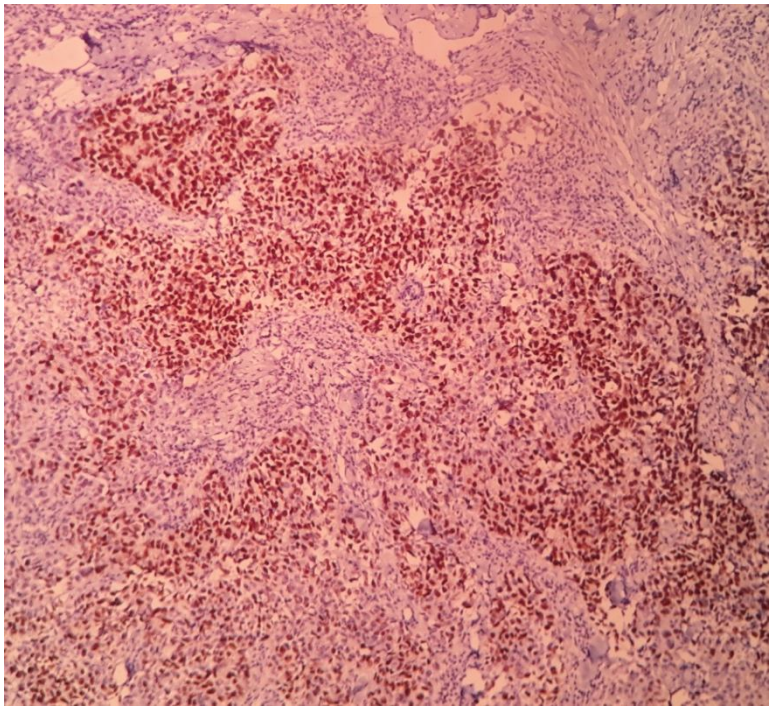
10x



40x

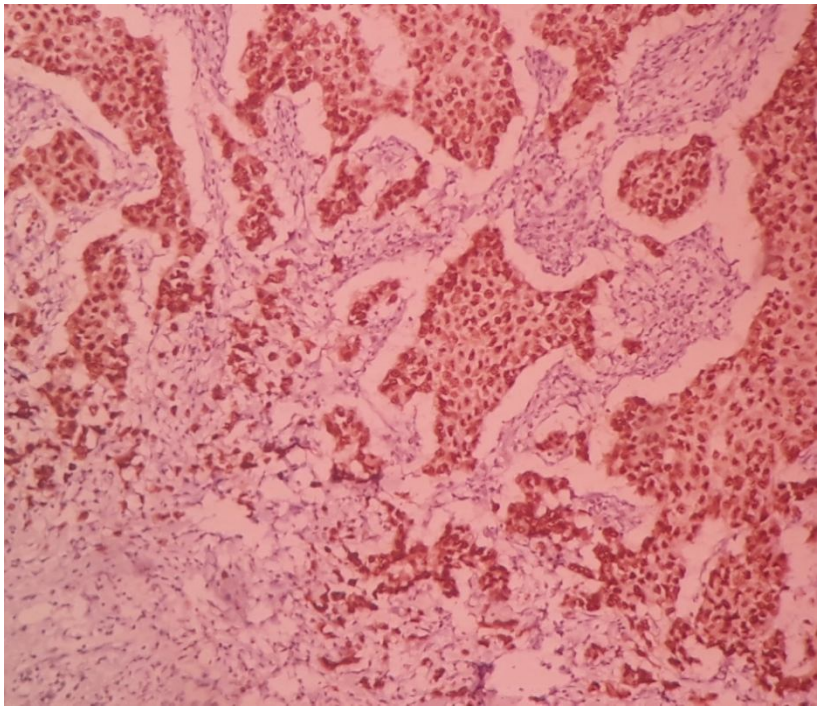


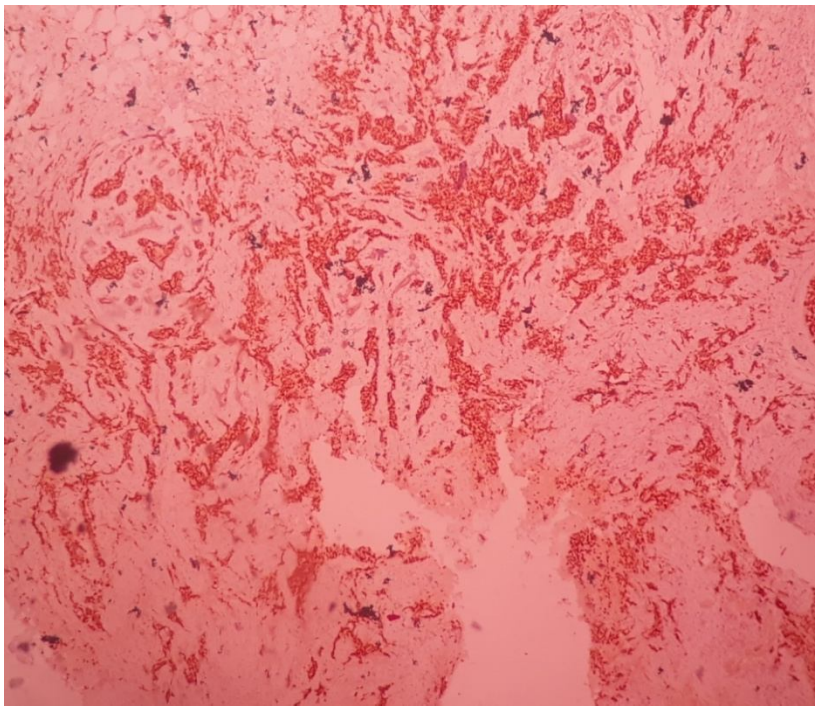
ER: showing positive staining in 80% of tumor cells



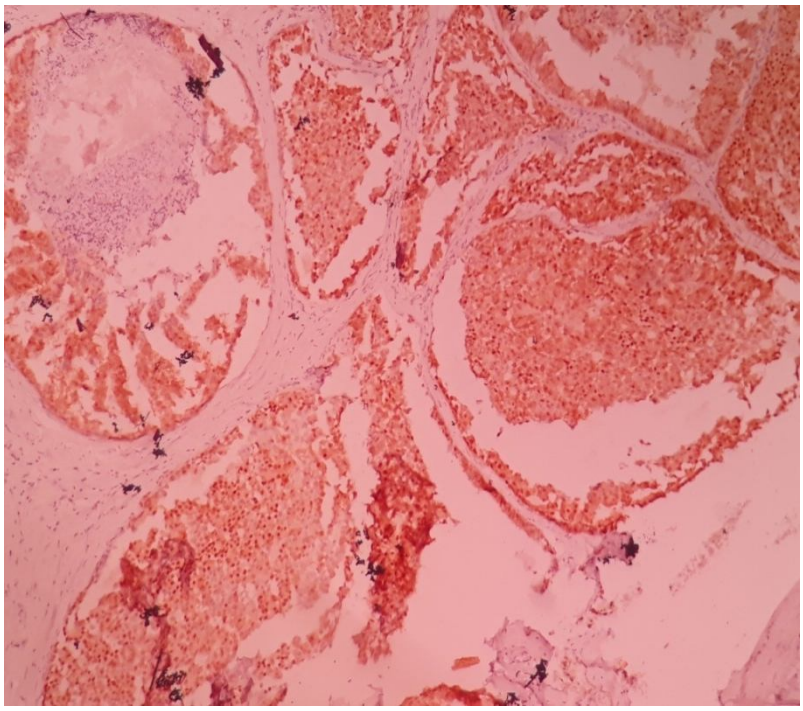
PR : Positivity in 80 % of tumor cells

GATA 3 : It is a nuclear marker, 70 % tumor cells shows positive staining.

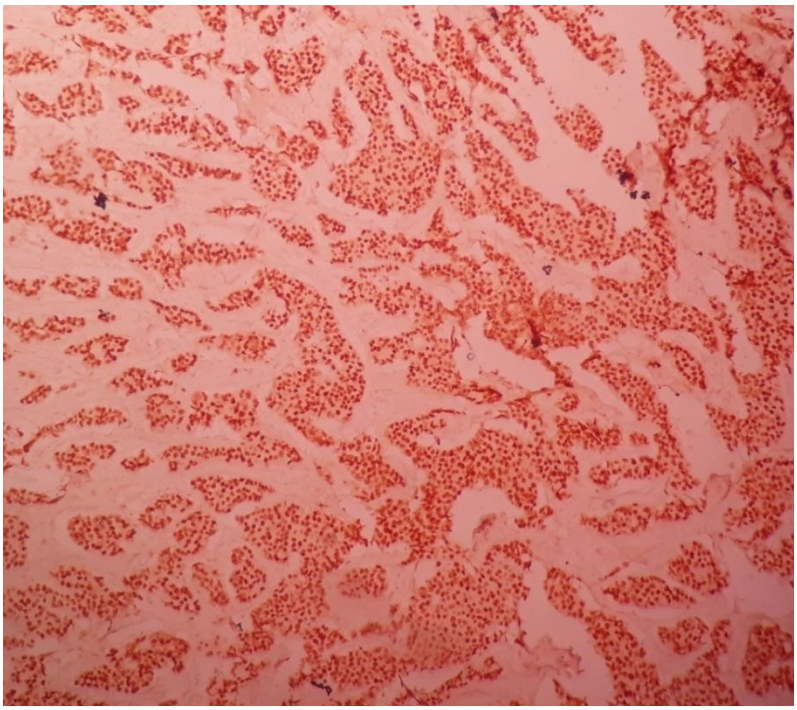




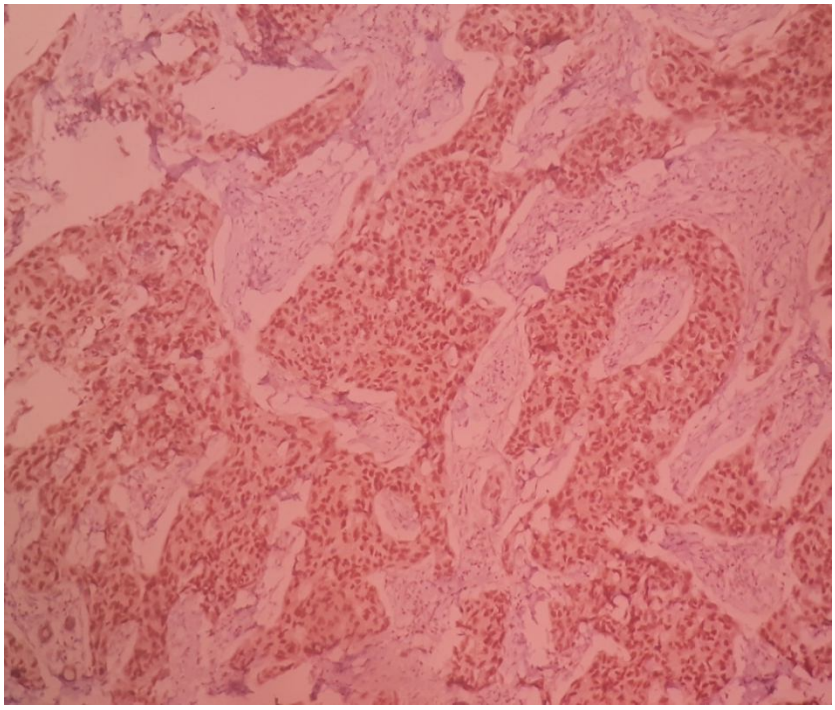
GATA3: staining 60 % of the tumor cells . Score (3+)



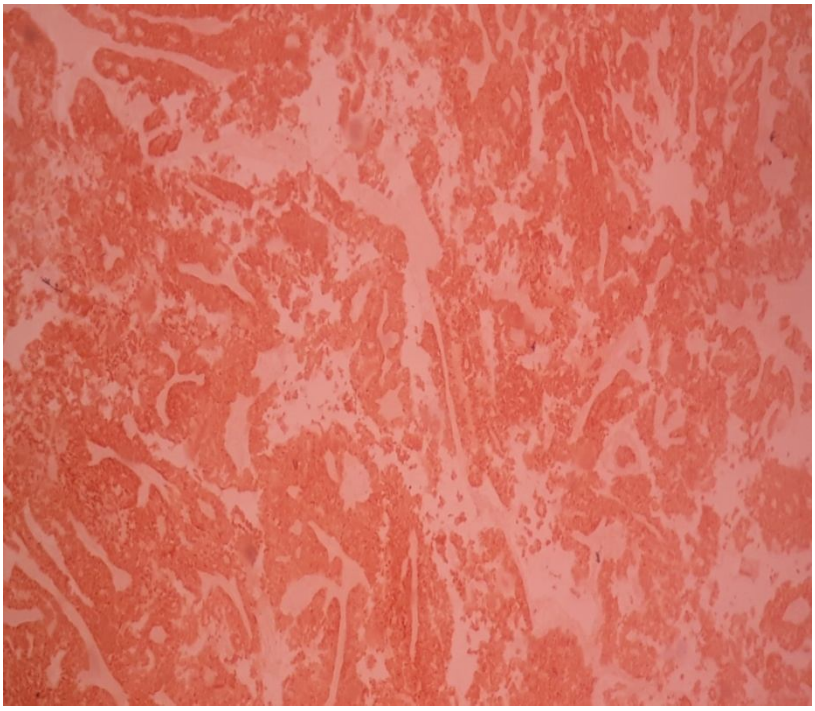
GATA3 : Staining 90% of the tumor cells (score 4+)



GATA3 : staining 90% of the tumor cells .(score 4+)



GATA 3 staining 80% of the tumor cells. Score (4+)



GATA 3 staining more than 90 % of the tumor cells

DISCUSSION

Invasive breast carcinoma is the most common carcinoma among women. According to World Health organization 22% of the female cancer cases are Carcinoma of breast. [95]

According to the Indian cancer registry data Ca breast is the most common malignant tumor among the Indian women. In India, the rate of incidence is 25.8 per 100,000 women. Mortality from breast cancer is higher in rural areas than urban areas of India. [3] Carcinoma of breast is rare in women below 25 years of age but, after 30 the incidence increases rapidly.**[94]**

Breast carcinomas with ER and PR positive status have excellent response to hormonal therapy but HER-2 positive cases are associated with poor prognostic outcome. Now-a-day's immunohistochemical analysis is necessary for proper diagnosis, prediction of prognosis and management of breast carcinoma.

GATA is a transcription factor that regulates the lineage of differentiation of many tissue types. GATA-3 is responsible for differentiation of luminal epithelial cells of breast and development of normal breast. Studies show that recurrent somatic mutation of GATA 3 gene is responsible for development of human breast cancers .**[96]**

It is expected that GATA-3 expression may have prognostic value in invasive breast carcinoma management. Some published literature has proposed that GATA-3 expression is limited to carcinoma of breast and urinary bladder. Therefore GATA-3 may be a sensitive and specific marker for metastatic breast carcinoma cases.[96,98]

In this study we evaluated the status of GATA3 expression in randomly selected Breast carcinoma specimens. Our sample size was 50 cases of invasive breast carcinoma with already known estrogen receptor (ER), progesterone receptor (PR) and HER 2 status.

Among these 50 breast carcinoma cases 49 female and one male patient of invasive breast carcinoma. The patient was 55 years male histologyically diagnosed as invasive breast carcinoma – no special type, Grade II. Though carcinoma in male breast is a very rare, but as being a tertiary referral center, it is occasionally encountered in our institute.

TYPE OF SPECIMEN RECEIVED:

Among the 50 specimens 45 cases were MRM (modified radical mastectomy), two cases of trucut biopsy, one breast conservative surgery, one wide local excision and one excision biopsy.

AGE WISE INCIDENCE OF CARCINOMA BREAST:

From our study we experienced that breast carcinoma below the age of 30 years is a rare finding. After 30 years the incidence is increases with age . We found that the peak incidence of breast carcinoma is 41 to 50 years of age. Majority of the patients of breast carcinoma are above the age of 40 years. The age wise incidence is relevant to other studies conducted in this field.

EXPRESSION OF GATA3:

In our study out of 50 cases 36 (72%) showed GATA 3 reactive and 14 cases (28%) cases were non reactive to GATA 3. Among the 36 positively stained 6 cases scored 1+, 5 cases scored 2+, 11 cases scored 3+ and 14 cases scored 4+ . (The Scoring of GATA3 is based on percentage of neoplastic cells taking the stain). In a study on GATA 3 expression in Breast carcinoma conducted at the Depertment of Pathology , The John Hopkins Hospital, Baltimore showed that overall in 67 % of breast carcinoma cases express GATA 3.[96] Another study conducted at Osaka University Graduate School of Medicine Showed that 74% of the Breast carcinoma cases expresse GATA3.[99]

GATA3 EXPRESSION WITH AGE

In this study the distribution of GATA 3 expression is showing in all age groups. Similarly, GATA 3 nonreactivity is also present in all age groups among the study population. We divided the positive cases into 4 categories , Score 1+ = 6% to 25 % cells positive, Score 2+ = 26% to 50% cells Positive. Score 3+ = 51% to 75% cells Positive. and Score 4+ = More than 75 % cells are positive. From the study we found some evidence that there is variable expression of GATA3 among each age group and its expression is independent of this variable.

GATA 3 EXPRESSION WITH GENDER:

Among all the 49 female patients 35 (71.42%) showed GATA 3 positivity. One male patient also showed reactivity to GATA 3.

On considering the gender with immunohistochemical expression of GATA3 ,both males and females showing positivity to the marker. As the distribution of cases among males and females is not uniform, it is not possible to come to a conclusion of relationship between GATA 3 expression and gender.

GATA3 EXPRESSION WITH TUMOR SIZE MAXIMUM DIAMETER:

In this study, the size of the tumor is grouped into 4 ranges – 1st group less than equal to 2.5 cm in maximum diameter ,second group 2.6 to 5 cm, third 5.1 to 7.5 cm and forth group 7.6 cm or more. Out of 50 cases expression of GATA 3 was found in all range of sizes 1st group (10 cases +ve) 2nd group (22 cases +ve), 3rd group (2 cases) and 4th group (2 cases). We experienced that there is variable expression of GATA 3 among each size range and its expression is independent of size of the tumor.

GATA 3 EXPRESSION WITH MICROSCOPIC VARIANT:

In our study majority of the cases were invasive breast carcinoma no special type(IBC-NST) contributing 47 cases. Other variants were papillary variant one case, medullary differentiation one case and apocrine differentiation representing one case. Total 34 (72.34%) cases of IBC –NST showed GATA 3 expression. Each case of papillary and apocrine variant also showed GATA 3 positivity. The medullary variant showed nonreactive to GATA 3. To establish a relationship between microscopic variant and GATA 3 expression a large number of study population is required.

GATA 3 EXPRESSION WITH MODIFIED BLOOM RICHERDSON GRADING SYSTEM FOR BREAST CARCINOMA:

According to histological grade the number of cases were Grade I (total 8 case), of grade II (total 36 cases) and Grade III (total 6 cases). In our study 7 cases (out of 8) grade I, total 26 cases (out of total 36 cases) grade II and 3 cases (out of total 6 cases) Grade III showed expression of GATA 3. It is evident that GATA 3 expression is independent of histological grade of the tumour.

GATA 3 EXPRESSION WITH MOLECULAR SUBTYPES, BASED ON ER, PR AND HER 2 NEU STATUS:

In our study population 22 (44%)luminal cases, 14 (28%) HER2 cases and 14 (28%) basal-like cases were seen.

GATA3 with luminal type :

Among the luminal subtype 91% (20 cases) showed GATA3 expression. Only 2 cases of luminal subtype (9%) showed nonexpression of GATA 3.

Out of the 20 GATA 3 positive cases of luminal type 6 cases scores 1+ expression, 3 cases (2+ expression), 5 cases (3+ expression) and 6 cases (4+ expression). From this study it is evident that most of

the luminal subtypes express GATA-3. The percentage of cells stained by the marker is variable.

GATA 3 expression with HER 2 :

In this study total 30 (60%) cases are HER 2 positive and 20 (40%) cases are HER 2 neu negative. Out of 30 HER 2 positive cases, 25 (83.33%) showed GATA 3 reactivity, 5 (16.66%) cases were GATA3 negative. Among the 20 HER-2 negative cases 11 (55%) showed positive to GATA 3, and 9 (45%) showed negative reaction to GATA 3.

From this data, the study suggested that GATA 3 expression is independent with the status of HER2 reactivity.

GATA 3 EXPRESSION IN (BASAL LIKE) TRIPLE NEGATIVE CASES

Among these cases total 14 cases were non reactive to ER, PR and HER 2 (basal like). Out of these, 8 (57%) cases show non reactivity to GATA3 also. The remaining 6 (42.85%) triple negative cases show GATA3 expression. From this data, it is evident that breast carcinoma may express GATA 3 in triple negative cases also.

SUMMARY

- Breast carcinoma is seen most commonly in females. Carcinoma of male breast is rare finding.
- Carcinoma of breast below the age of 30 years is uncommon. After 30 years the incidence increases. In our study maximum number of cases seen at 4^{th} decade of life.
- Majority of the breast carcinoma cases (72%) show GATA3 expression . Only 28% of breast carcinoma cases show negative reaction to GATA 3 .
- Immunohistochemical expression of GATA 3 in breast carcinoma is not affected by gender.
- Variable expression of GATA3 is seen among each age group. GATA3 expression is independent of the age.
- We experienced that there is variable expression of GATA 3 among each size range. Expression of GATA 3 is independent of the size of the tumor.
- 72.34% cases of invasive breast carcinoma no special type (IBC –NST) showed GATA 3 expression.
- Each case of Papillary and apocrine variant also showed GATA 3 positivity.

- The medullary variant showed nonreactivity to GATA3. To establish a relationship between microscopic variant and GATA3 expression, a large number of study population is required.
- GATA3 expression is independent of histological grade of the tumour.
- Molecular subtypes, based on ER, PR and HER2 luminal type 44%, HER2 type 28% and basal-like (triple negative) 28% cases were seen.
- Among the luminal subtype 91% showed GATA3 expression.
- The percentage of cells stained by the marker is variable.
- Among the HER-2 negative cases, 55% showed positive to GATA 3, and 45% showed negative reaction to GATA 3. Our study suggests GATA 3 expression is independent with the status of HER2 reactivity.
- Total 57% basal like cases show non reactive to GATA3 also. The remaining 42.85% triple negative cases show GATA3 expression. It is evident that breast carcinoma may express GATA 3 in triple negative cases also.

CONCLUSION

It is a hospital based study and it may not represent the true incidence of the disease in the community. Most of the patients of breast carcinoma are above 40 years of age. Carcinoma of breast under 30 years is seen rarely. Majority of the breast carcinoma cases shows GATA3 expression . Immunohistochemical expression of GATA 3 in breast carcinoma is not affected by gender, age, size of the tumor and histological grading . To establish a relationship between microscopic variant and GATA3 expression a large number of study population is required. Among the luminal subtype, 91% shows GATA3 expression with variable percentage of cells stained case by case. GATA 3 expression is independent with the status of HER2 reactivity. GATA3 expression in triple negative cases is 42.85% .

From the study it is found that GATA3 may not be shown to be a prognostic factor, but majority of the GATA 3 expressing tumors are luminal type which show excellent response to hormonal therapy. GATA 3 expression in triple negative tumors may be an important clue to establish this marker for metastatic breast carcinoma (MBC). Further studies are needed to know the role of GATA 3 in breast carcinomas.

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George Yang, M.D.,1 Mike Lacey, M.D.,1 Kelsea Cumminings, B.S.,1 Ourhay Mego, B.S.,1 Ankur R Sangoi, M.D.,2 Bijayee Shretha, M.D., Ph.D.,2 Andrew H. Beck, M.D., Ph.D.3

1Cell Marque, Rocklin, CA; 2El Camino Hospital, Mountain View, CA; 3Department of Pathology, Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, MA

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INFORMATION SHEET

- We are conducting a study on GATA 3 expression in breast carcinoma and its association ER, PR, Her 2 neu status among patients attending Rajiv Gandhi Government General Hospital, Chennai and for that your sample may be valuable to us.
- The purpose of this study is to plan for to understand GATA 3 IHC marker expression in breast carcinoma and its association ER, PR, Her 2 neu status.
- We are selecting certain samples with reduced platelet count and we may be using your sample to perform tests which in any way do not affect your final report or management.
- The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.
- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.
- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of investigator

Signature of participant

Date

ஆராய்ச்சி தகவல் தாள்

ஆராய்ச்சி தலைப்பு : **மார்பக புற்றுநோய் காட்டா 3 (GATA 3) புற்றுநோயின் வெளிப்பாடு மற்றும் ஈ.ஆர், பி.ஆர், ஹெர் 2 நியூ (ER, PR, HER 2 neu) புற்றணுக்களோடு உள்ள அதனுடைய தொடர்பு பற்றிய ஓர் ஆய்வு.**

ஆய்வாளர் : மரு. ரிட்டுபர்னா புர்வா,
இரண்டாம் ஆண்டு,
நோய்குறியியல் துறை,
சென்னை மருத்துவக் கல்லூரி, சென்னை - 600003.

தங்களது மார்பக புற்றுநோய் கட்டி (அறுவை சிகிச்சை செய்யப்பட்ட கட்டி) இங்கு பெற்றுக் கொள்ளப்பட்டது.

சென்னை அரசு பொது மருத்துவமனைக்கு வரும் நோயாளிகளிடம் இருக்கும் மார்பகப் புற்றுநோய் கட்டிகளைப் பற்றிய ஒரு ஆராய்ச்சி இங்கு நடைபெற்று வருகின்றது.

மார்பக புற்றுநோய் காட்டா 3 (GATA 3) புற்றுநோயின் வெளிப்பாட்டினை சில சிறப்புப் பரிசோதனைகளின் மூலம் எளிதில் கண்டுபிடித்து ஆராய முடியும் என்பதே இந்த ஆராய்ச்சியின் நோக்கமாகும்.

நீங்களும் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்புகிறோம். இந்த ஆராய்ச்சியில் உங்களுடைய திசுக்களை எடுத்து சில சிறப்புப் பரிசோதனைக்கு உட்படுத்தி அதன் தகவல்களை ஆராய்வோம். அதனால் தங்களது நோயின் ஆய்வறிக்கையோ அல்லது சிகிச்சையோ பாதிப்புக்குள்ளாகாது என்பதையும் தெரிவித்துக் கொள்கிறோம்.

முடிவுகளை அல்லது கருத்துகளை வெளியிடும் போதோ அல்லது ஆராய்ச்சியின் போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிட மாட்டோம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில் தான் இருக்கிறது. மேலும் நீங்கள் எந்நேரமும் இந்த ஆராய்ச்சியில் இருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த சிறப்புப் பரிசோதனைகளின் முடிவுகளை ஆராய்ச்சியின் போது அல்லது ஆராய்ச்சியின் முடிவின் போது தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த ஆய்வை பற்றிய சந்தேகங்களுக்கு தொடர்பு கொள்ள வேண்டியவர் : மரு. ரிட்டுபர்னா புர்வா, செல் : 9600024026

பங்கேற்பாளர் கையொப்பம்..... இடம் :..... தேதி :.....

பங்கேற்பாளர் பெயர் மற்றும் விலாசம் .....

ஆராய்ச்சியாளர் கையொப்பம்..... இடம் :..... தேதி :.....

INFORMED CONSENT FORM

Title of the study: **"GATA 3 EXPRESSION IN BREAST CARCINOMA, AND ITS ASSOCIATION WITH ER, PR, HER 2NEU STATUS – A 3 YEARS STUDY IN A TERTIARY CARE CENTRE"**

Name of the Participant :
Name of the Principal (Co-Investigator) :
Name of the Institution : MadrasMedicalCollege
Name and address of the sponsor / agency (ies) (if any) :

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in **"GATA 3 expression in breast carcinoma, and its association with ER, PR, HER 2neu status – a 3 years study in a Tertiary Care Centre"**

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study in which paraffin embedded tissues will be processed on the light microscopy and Immunohistochemistry study.
4. I have been explained about my rights and responsibilities by the investigator. I have the right to withdraw from the study at any time.
5. I have informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
7. I have understand that my identity will be kept confidential if my data are publicly presented
8. I have had my questions answered to my satisfaction.
9. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature / thumb impression of the participant (or legal representative if participant incompetent)

Name _____ Signature_____ Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____ Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____ Date_____

ஆராய்ச்சி ஒப்புதல் கடிதம்

ஆராய்ச்சி தலைப்பு : **மார்பக புற்றுநோய் காட்டா 3 (GATA 3) புற்றுநோயின் வெளிப்பாடு மற்றும் ஈ.ஆர், பி.ஆர், ஹெர் 2 நியூ (ER, PR, HER 2 neu) புற்றணுக்களோடு உள்ள அதனுடைய தொடர்பு பற்றிய ஓர் ஆய்வு.**

சென்னை மருத்துவக் கல்லூரி நோய்குறியியல் துறையில் மரு. ரிட்டுபர்னா புர்வா அவர்கள் மேற்கொள்ளும் இந்த ஆய்வில் பங்குகொள்ள ..... ஆகிய நான் முழு மனதுடன் சம்மதிக்கிறேன்.

எனக்கு விளக்கப்பட்ட விஷயங்களை நான் புரிந்து கொண்டு நான் எனது சம்மதத்தைத் தெரிவிக்கிறேன்.

இந்த ஆராய்ச்சியில் பிறரின் நிர்ப்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில் தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின்வாங்கலாம் என்பதையும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் நான் புரிந்து கொண்டேன்.

நான் மார்பக புற்றுநோயில் ஏற்படும் காட்டா 3 (GATA 3) நோயினை குறித்த இந்த ஆராய்ச்சியின் விவரங்களைக் கொண்ட தகவல் தாளைப் பெற்றுக் கொண்டேன்.

நான் என்னுடைய சுயநினைவுடன் மற்றும் முழு சுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக் கொள்ள சம்மதிக்கிறேன்.

எனக்கு அறுவை சிகிச்சை செய்யப்பட்டு நோய்க்குறியியல் துறையில் திசுப் பரிசோதனைக்கு பயன்படுத்தப்படும் திசுவினை மெழுகுக்கட்டிகளை வைத்து ஆராய்ச்சி மற்றும் சிறப்புப் பரிசோதனை செய்து கொள்ள சம்மதம் தெரிவிக்கிறேன்.

பங்கேற்பாளர் கையொப்பம்..... இடம் :..... தேதி :.....
பங்கேற்பாளர் பெயர் மற்றும் விலாசம் .....

ஆராய்ச்சியாளர் கையொப்பம்..... இடம் :..... தேதி :.....

BX	age	sex	GATA-3	IHC No	ER	PR	HER 2 NEU	clin	Proce	size of thu	type micr	microscopic GF	LN metasta	TNM	BIRADS
271/18	40	F	4++ve	53/18	NEG	neg	1+		MRM	3X2X2	IBC-NST	II	NO	T2N0M0	
286/18	50	F	4++ve	171/18	NEG	neg	3+		WIDE	3X1.8X1.6	IBC-NST	II		T2N0M0	
293/18xxx	35	F	2++ve	XXX	NEG	10%(5)	NEG		MRM	9X8X3	IBC-NST	I	2/5 NODEs	T2N1M0	
303/18xxx	45	F	neg	215/18	NEG	neg	NEG		MRM	2X1	IBC-NST	II	3/5 NODE	T2N1M0	IV= 4
304/18	47	F	3++ve	108/18	70%(5+3)	40%(4+3)	NEG		MRM	4X3X2	IBC-NST	II	2/8 NODE	T2N1M0	
663/18	64	F	4++ve	206/18	65%(4+3)	65%(4+3)	2+		Mast	2.5x2.5x1.5	IBC-NST	II		T2N0M0	
909/18	46	F	4++	138/18	90%(5+3)	10%(2+2)	2+		MRM	2X2X2	IBC-NST	I	N1	T1cN1M0	
910/18	36	F	1++ve	201/18	15%(3+2)	neg	1+		MRM	2.5X2.3X1.	IBC-NST	II	N0	T2N0M0	
1257/18	44	F	4++ve	249/18	80%(5+3)	4+3	3+		MRM	6X5X2	Invasive b	II	N3	T3 N3 M0	
1311/18	37	F	3++ve	231/18	neg	neg	3+		MRM	4x3.5x2.5	Invasive b	III	N0	T2N0M0	
1380/18	71	F	1++ve	225/18	90%(5+3)	30%(3+4)	3+		TRUC	0.2	IBC-NST	II			
1506/18 xxx	37	F	1++ve	240/18	65%(4+3)	65%(4+3)	3+		MRM	6.5X4.5X2	IBC-NST	III	N3	T3N3aM0	
1587/18xxx	38	F	neg	187/18	NEG	neg	3+		BREA	1X1X1	IBC-NST	III	N0	T1bN0M0	
1669/18	36	F	4+VE	195/18	NEG	NEG	NEG		MRM	3X3X2.5	IBC-NST	II	N0	T2N0M0	IV= 4
1730/18	48	F	4+VE	204/18	NEG	NEG	2+		MRM	4.5x3.5x3	IBC-NST	I	N0	T2N0M0	
1877/18	60	F	4 +VE	254/18	NEG	neg	2+		MRM	10X6X2.5	IBC-NST	II	N0	T3N0M0	
2438/18	37	F	3+	285/18	NEG	neg	2+		MRM	3X2X2	IBC-NST	II	N0	T2N0M0	
2489/18	56	F	4 +VE	307/18	NEG	neg	3+		MRM	3X2.5X2	IBC	I	N0	T2N0M0	
2525/18	52	F	NEG	317/18	NEG	neg	NEG		MRM	4X3.5X3	IBC-NST	III	NO	T2N0M0	
2684/18	60	F	4++ve	338/18	80%(5=3)	30%(3+2)	2+		MRM	3X3X2	IBC-NST	II	N0	T2N0M0	
2730/18	59	F	3+	339/10	NEG	neg	NEG		MRM	3X2X2	IBC-NST	I	N0	T2N0M0	
2792/18	60	F	4++ve	309/18	90%(5+3)	90%(5+3)	3+		MRM	2.5X2.5X2.	INVACIVE	II	N0	T2N0M0	
3516/18 XX	45	F	4+VE	394/18	NEG	neg	NEG		MRM	NE WELL D	IBC-NST	II	1 NODE +v	T2N1aM0	
3633/18	42	F	2 +VE	507/18	NEG	neg	3+		MRM	2X2X1.5	IBC-NST	II	N1	T1cN1M0	
3893/18	46	F	neg	433/18	NEG	neg	2+		MRM	4X4X3.5	IBC-NST N	II	NO	T2N0M0	
4117/18**	40	F	3++ve	476/18	NEG	neg	NEG		MRM	4X1.5X1.5	IBC-NST	II	1 NODE +v	T2N1aM0	
4121/18**	29	F	3+VE	450/18	90%(5+3)	60%(4+3)	3+	fibr	excis	3x2x1	IBC-NST	II	0	T1N0M0	
9099/16	58	F	1++ve	950/16	90%(5+2)	6/8(4+2)	NEG		mrm	3x2x1.5	IBC-NST	II		T2N0M0	
9535/16	45	F	2++ve	1005/16	NEG	3/8+VE	NEG		MRM	5x2x2	IBC-NST	II		T4bN0M0	
2081/17	45	F	neg	250/17	90%(5+3)	60%(4+3)	3+		MRM	2.5x2x1.5	IBC-NST	II		T2N0M0	
936/17	48	F	4+++ve	420/17	25%(3+2)	neg	3+		mrm	3X1.8X1.5	IBC-NST	III		T2N0M0	
971/17	65	F	2++ve	160/17	NEG	neg	3+		MRM	2.5x2.5x2.5	IBC-NST	I		T2N0M0	
987/17	55	M	1++ve	239/17	90%(5+3)	90%(5+3)	2+		MRM	3x2x1	IBC-NST	II		T3N0M0	
3489/17	65	F	neg	460/17	NEG	neg	NEG		MRM	4x2x1	IBC-NST	II		T2N0M0	
4603/17	40	F	neg	551/17	NEG	neg	2+		MRM	2.5X2.5X2.	IBS-NST	II		T2N0M0	
9669/16	42	F	3++ve	1023/16	NEG	neg	NEG		MRM	3x2x2	IBC-NST	II		T3N0M0	
5217/16 xxx	52	F	neg	540/16	NEG	neg	3+		mrm	4x2x1	IBC-NST	II		T2N0M0	
6231/16	55	F	3++ve	627/16	7/8+VE	6/8+VE	NEG		MRM	3.5x2x1.5	IBC-NST	II		T2N0M0	
7894/16xxx	29	F	neg	838/16	NEG	neg	NEG		MRM	2.5x2.5x1.5	IBC-NST	III		T2N0M0	
7932/16xxx	44	F	neg	874/16	NEG	neg	NEG		MRM	3.5x2x1.5	IBC-NST	II		T2N0M0	
7988/16	65	F	2++ve	846/16	3/8+VE(1+2)	neg	2+		MRM	4x2.5x2.5	IDC-NST	I		T2N0M0	
8247/16	46	F	3++	854/16	6/8+VE(3+3	5/8+VE	3+		MRM	4.5x3x2	IBC-NST	II		T2N0M0	
917/17???	20	F	3++ve	104/17	3+2	2+2	3+		mrm	3x1.5x1.5	IBC-NST	II		T2N0M0	
9486/16	58	F	3++ve	1028/16	NEG	neg	3+		MRM	3.5x2.5x2.5	IDC-NST A	II		T2N0M0	
9677/16	58	F	1++ve	1103/16	NEG	5/8(2+3)	3+		MRM	3.5x2x1	IDC-NST	II		T2N0M0	
10612/16	48	F	neg	1106/16	NEG	POSITIVE	NEG		mrm	4x2x1	IDC-NST	II		T2N0M0	
5276/16	43	F	4++ve	565/16	NEG	neg	NEG		MRM	2x1x1	IDC-NST	II		T2N0M0	
6680/16??	41	F	neg	697/16	NEG	NEG	NEG		mrm	3x2.5x1	IDC-NST	II	T3N1M0	T3N1M0	
1516/16	48	F	NEG	151/16	NEG	neg	NEG		TRUC	3x3x1	IBD-NST	II	XX	T2N0M0	
1716/16	56	F	NEG	209/16	NEG	neg	NEG		MRM	2.5x2x1.7	IDC-NST	I		T2N0M0	